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Soil matters: impacts of farming practices on natural regulation of root pests of field vegetables.

Céline Marie Coralie DELABRE

Doctor of Philosophy - the University of Edinburgh - 2020

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A Mamy,

à qui je pense souvent et qui aurait été fière,

Toujours pas de prêtre dans la famille, mais un docteur, c'est déjà ça.

Declaration

I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted for any other degree or processional qualification except as specified.

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Abstract

Agricultural intensification and over-reliance on pesticides have had serious detrimental effects on ecosystems. Agroecology research is attempting to restore healthier agroecosystems, capable of delivering a wide range of ecosystem services, including natural regulation of crop pests by their natural enemies. This study explores the impacts of farming practices on the cabbage root fly *Delia radicum*, whose larvae cause serious damage to brassica crops. Two long term experimental rotations, comparing organic and conventional practices, were monitored over two seasons for fly eggs and pupae as well as their natural enemies. Additionally, a paired soil survey was conducted across the UK and Ireland to investigate varied commercial organic and conventional practices, their impact on soil as habitat supporting plant growth, as well as on soil-based natural regulation.

Organic practices in the experimental rotations had an overall positive impact, reducing fly eggs in both sites and pupal numbers in one site, compared to pesticide treated plots, as well as increasing activity density of their potential epigeal and belowground predators. Experimental field soils were also used to further investigate the potential regulation through insect pathogens using a model pest, but no consistent significant differences between management types were detected. Additionally, potential bottom up control through plant-soil-pest interactions was also tested by growing brassica plants in those same soils whilst inoculating them with *D. radicum* eggs. Too few pupae were extracted to be able to conclude decisively, but data did not point towards a reliable enhanced pest suppression in organic soils.

Paired commercial soils were impacted by local management, with organic soils being more biologically active. Unlike experimental field soils, organic commercial soils were more suppressive overall for the model pest, potentially due to entomopathogenic nematode presence. Brassicas grown in organic commercial soils developed significantly larger root systems, without a reduction of top biomass, but inoculation experiments once again did not reveal any clear difference in pest survival between organic and conventionally managed soils.

In line with current research, chemically based management was shown to have a detrimental effect on soil biological activity and pest antagonist communities when compared to organic management. Under the adequate management, soil can help foster

functional biodiversity to help effectively deliver a wide range of ecosystem services including pest regulation. As a habitat, soil is unfortunately often overlooked in conservation biocontrol studies, even though it is an integral part of sustainable and resilient agroecosystems. This thesis attempts to highlight the importance of also including soil in natural pest regulation studies along with aboveground landscape elements that are more easily manipulated.

Lay summary

This thesis investigates the impacts of contrasting organic and conventional farming practices on the soil, the insect pest the cabbage root fly, and its natural enemies. As our food production systems need to become more sustainable to reduce their negative impacts while producing enough to feed a growing population, farmers need viable alternatives to chemical pesticides to manage insect pests. Beneficial organisms naturally present in the field can contribute to reducing the impact of these pests. However, these beneficial organisms can be negatively impacted by farming practices other than the use of synthetic pesticides. Using the cabbage root fly as an example of a damaging root pest that farmers still struggle to fight, we assessed the impacts of conventional and organic farming practices on the pest and its natural enemies, as well as on the soil itself, where the damaging stage of the pest resides. Using experimental fields as well as commercially managed fields, we show that organic management had an overall positive impact on the soil, the brassica crop and the natural enemies of the cabbage root fly, as the pest incidence was reduced overall. We included the common natural enemies of the fly as well as the smaller, less studied ones, which clearly had an impact. By focussing on the soil, this thesis highlights the importance of this large piece of the agricultural landscape when considering sustainable pest management.

Chapter 1 General introduction

1.1 Transitioning towards sustainable food systems

The need for sustainable agriculture globally

Global sustainable food security remains a challenge for humanity. Even if modern intensive agriculture has been able to produce enough to feed a rapidly growing population (FAO and WHO, 2009; Hazell et al., 2008), its negative impacts have crippled agroecosystems globally (Pretty et al., 2000; Tilman et al., 2002; Foley et al., 2011). Those negative impacts includes serious biodiversity loss (Cardinale et al., 2012; Hooper et al., 2012; Krebs et al., 1999; Pretty et al., 2000), oversimplification of agricultural landscapes (Tscharntke et al., 2005), and soil degradation (Amundson et al., 2015; Borrelli et al., 2017; FAO, 2015a; Montgomery, 2007; Robinson et al., 2017). The turn of the 21st century saw research and policy both strongly advocate for a transition towards sustainable agriculture to improve global food security, also taking into account resource constraints and climate change (European Union, 1997; FAO and WHO, 2009; Gliessman et al., 1998; IAASTD, 2009; Krebs et al., 1999; Ruttan, 2015; Tilman, 1999).

Through sustainable intensification

We are now seeing signs of this transition (Garibaldi et al., 2017; Gunton et al., 2016; Pretty et al., 2014), in part through sustainable intensification. Sustainable intensification aims at increasing agricultural productivity whilst enhancing ecosystem services that regulate and support food production (Bommarco et al., 2013; Charles et al., 2014; Rockström et al., 2017; Tittonell, 2014). Also referred to as ecological intensification but not always equivalent (Tittonell, 2014) and somewhat ill-defined or without practical guidelines (Gunton et al., 2016; Petersen et al., 2015; Pywell et al., 2015; Wezel et al., 2015), this concept is nevertheless now part of recent international development policies such as the UN's Sustainable Development Goals for 2030 (UN, 2015) and the European Union Farm to Fork strategy (European Commission, 2020), aiming at establishing a new agricultural paradigm (Garibaldi et al., 2019). Intensively managed agroecosystems can be modified, using either efficiency and substitution tools, or actual system redesign (Pretty et al., 2018; Wezel et al., 2014) in order to harness and combine diverse ecosystem services to recreate

multifunctional and stable agricultural habitats (Bretagnolle et al., 2018; Dainese et al., 2019; Firbank et al., 2013; Lefcheck et al., 2015; Tittonell, 2014).

The agroecology framework

To be implemented successfully, this transition in land use cannot only be ecological in nature and requires a shift through the entire food system. Agroecology, the application of ecological concepts and principles to the design and management of sustainable agroecosystems (Altieri, 1995; Gliessman, 1990), is in part guiding this ecological transition (Altieri et al., 2015, 2011; FAO, 2016; Wezel et al., 2014) by also integrating the indispensable agronomic, economic and social dimensions of agriculture (Douwe van der Ploeg et al., 2019; Francis et al., 2003; Wezel et al., 2009). As an integrated approach, agroecology has been identified as a key tool for global food security, by fostering co-creation of knowledge and implementation of sustainable farming practices by farmers themselves, whilst ensuring rural communities prosper (Dalgaard et al., 2003; De Schutter et al., 2011; Ernesto Méndez et al., 2013; FAO, 2016; Gliessman, 2015; Holt-Giménez et al., 2013; IPES-Food, 2015; Lescourret et al., 2015). Whilst tightly focused on the specific problem of root pests in field vegetables, this thesis draws from agroecological concepts and uses agroecology as a framework throughout, advocating the maintenance or enhancement of biological integrity and diversity in agroecosystems (Altieri, 1999) in the context of sustainable intensification.

1.2 Conservation biocontrol in the Integrated Pest Management context

Integrated pest management for sustainable intensification

Negatively impacting food security, insect pests continue to cause significant losses globally, estimated at least $470 billion per annum (Culliney, 2014). Concurrently, modern chemical pest control has led to serious persistent negative impacts on ecosystems, including loss of biodiversity, and increased pollution and pest resistance (Geiger et al., 2010; Hillocks, 2012; Kogan, 1998; Lewis et al., 1997; Tilman, 1999). Chemical pesticide availability is declining due to regulations becoming more stringent (Butler, 2018), as health costs to farmers (Lopes Soares et al., 2009; Maroni et al., 2006) and public health concerns

(Pimentel, 2005; Wilson et al., 2001) are being highlighted. In this context, a more systemic approach to crop protection started to emerge in the second part of the 20th century in order to reduce overreliance on synthetic pesticides (Kogan, 1998). Integrated Pest Management (IPM) combines prevention, monitoring and protection in order to keep pest impacts under economic thresholds (Kogan, 1998; Pimentel et al., 2014) and has been identified as a key tool for sustainable intensification globally (Pretty and Bharucha, 2015; Reeves et al., 2016; Shields et al., 2019). In Europe, the application of its principles is now a requirement (European Parliament, 2009) and member states are required to develop national action plans for pesticide reduction (Barzman et al., 2011). IPM relies on the combination of a wide range of synergistic practices including crop rotations, use of resistant varieties, pest forecasting and monitoring, and manipulating planting times, focussing on non-chemical control methods (Barzman et al., 2015; Stenberg, 2017). Implementing IPM practices has been linked to many benefits including yield increases of 5–40% and declines in pesticide use of 30–70% (Pretty et al., 2018).

Natural regulation of pests for IPM

Biological regulation of pests by natural enemies is at the heart of IPM (Barzman et al., 2015; Brewer et al., 2012; Luck et al., 1988; Stern et al., 1959) and the protection and enhancement of beneficial organisms has been clearly identified as one of the six principles of IPM described in the Annex III to the Sustainable Use of Pesticides Directive (European Parliament, 2009). Thought to be responsible for the suppression of up to 50% of pests (Pimentel, 2005), this natural pest regulation was central to crop production before the introduction of synthetic pesticides during the Green revolution (Begg et al., 2017; Gurr et al., 2015) and as an ecosystem service has been valued at $4.5 billion annually (Losey et al., 2006). However, entomofauna has been in strong decline, amongst other taxa but with a greater extinction rate, due to widespread habitat loss and pesticide use in modern agriculture (Firbank et al., 2008; Hallmann et al., 2017; Sánchez-Bayo et al., 2019; Thomas et al., 2004), seriously impacting insect ecosystem services including pest regulation. As ecological intensification is aiming to restore and enhance ecosystem services supporting sustainable food production, natural pest regulation has benefited from renewed interest from both research and policy (Gurr et al., 1998) through the discipline of conservation biological control (CBC).

Conservation biological control to enhance natural regulation of pests

Conservation biological control includes a wide range of management practices aiming at the conservation and enhancement of pest natural enemies (in terms of abundance and diversity) for pest management (Gurr et al., 2004; Jonsson et al., 2008; Straub, Finke and Snyder, 2008; Rusch et al., 2010; Begg et al., 2016; Shields et al., 2019). In practice, it mainly focuses on aboveground habitat and vegetation manipulation techniques (Rusch et al., 2010; Shields et al., 2019), at local and landscape scales (Begg et al., 2017), in order to foster indigenous natural enemy abundance and diversity, as opposed to classic and inundation biocontrol, where natural enemies are artificially released into the agroecosystem (Bale et al., 2008; Gurr et al., 2015). Enhancing natural regulation of pests is of strong interest to policymakers, farmers and agronomists (Dicks et al., 2016) as conservation biological control has the potential to contribute to farm profitability by reducing input costs, increasing yields and opening markets where low pesticides residues are valued (Cullen et al., 2008). This discipline has evolved rapidly over the last two decades, from unoptimized seed mixes for flower strips to a more knowledge-intensive, ecological engineering approach (Gurr et al., 2004; Shields et al., 2019). Having been applied to various cropping systems in diverse climates, conservation biological control practices have been shown to generally conserve and enhance natural enemy presence, yet still fail to reliably deliver the expected enhanced pest control (Gurr et al., 2000a; Karp et al., 2018; Settele et al., 2018; Tscharntke et al., 2016) mainly due to the complexity of ecological processes and dynamics involved, leading farmers to having limited confidence in natural regulation as a reliable process (Zhang et al., 2018).

Conservation biological control is knowledge-intensive

To be successful, conservation biological control practices should be underpinned by sound ecological knowledge of both pest and natural enemies (Gurr et al., 2003b; Altieri and Nicholls, 2004; Gurr et al., 2004; Letourneau et al., 2009; Begg et al., 2016; Shields et al., 2019), as well as taking into consideration ecological community concepts such as complementarity of natural enemies (Perović et al., 2017; Snyder, 2019), or intraguild predation (Prasad et al., 2004; Rosenheim, 1998; Snyder et al., 2003; Tylianakis et al., 2010), as conservation biological control requires a multitrophic perspective (Tscharntke et al., 2007; Winkler et al., 2010; Holland et al., 2016; Pretty et al., 2018; Brévault and Clouvel, 2019). Concurrently, extensive knowledge on the impacts of those habitat manipulations and farming practices is also required to ensure successful pest suppression, both for local

management such as tillage (Mesmin et al., 2020; Roger-Estrade et al., 2010), intercropping and undersowing (Gurr et al., 2000b; Hooks et al., 2003; Wezel et al., 2014), or flower strips (Winkler et al., 2010; Holland, 2012; Nilsson et al., 2016; Gardarin et al., 2018) and landscape scale dynamics such as spill-over and dispersion from semi-natural habitats (Rand, Tylianakis and Tscharntke, 2006; Tscharntke et al., 2007; Holland et al., 2016; Bartual et al., 2019; Holland et al., 2020), or landscape composition and complexity (Bianchi et al. 2006; Tscharntke et al., 2007; Batáry et al., 2011; Chaplin-Kramer et al., 2011; Woltz, Isaacs and Landis, 2012; Vasseur et al., 2013; Jeanneret et al., 2016; Rusch et al., 2016; Karp et al., 2018).

1.3 Belowground habitat management: including soil in conservation biological control

The soil as missing puzzle piece

If successful conservation biological control requires extensive knowledge of the community ecology of the managed agroecosystem, as well as farming and manipulation practices' impacts, one extensive piece of the agroecosystem puzzle is too often overlooked by conservation biological control studies: the soil. Soil can contribute to pest regulation in two main ways. Contributing to top-down regulation, the soil can be both be a source and a supporting habitat for pest natural enemies (Campos-Herrera et al., 2013; Kaya et al., 1993; Klingen et al., 2007; Ratnadass et al., 2006; Roger-Estrade et al., 2010; Rusch et al., 2010; Villani et al., 1990). Concurrently, wider soil biodiversity can contribute to optimal plant health and help plants to defend themselves against pest attacks (Kupferschmied et al., 2013; Pieterse et al., 2014; Pineda et al., 2010; van Dam, 2009). Despite this pest regulation potential, soil function tends to only be mentioned and not developed in soil biodiversity research (Decaëns et al., 2006; Gardi et al., 2009; Lavelle et al., 2006; Lemanceau et al., 2014; Wagg et al., 2014), apart from the seminal agroecology work of Altieri and Nicholls (Altieri, Schmidt and Montalba, 1998; Altieri, 1999; Altieri and Nicholls, 2003, 2004; Altieri, Nicholls and Fritz, 2005; Altieri, Ponti and Nicholls, 2005; Altieri et al., 2015). The soil pest regulation potential has also seldom been mentioned in recent conservation biological

control reviews (see Gurr et al. 2003a; Begg et al., 2016; Gurr et al., 2017; Perović et al., 2017; Shields et al., 2019).

Soil health for sustainable intensification

More generally, similar to habitat manipulation studies, biodiversity loss accounting has mainly focussed on aboveground habitats even though a large part of the planet's biodiversity is actually belowground (Wagg et al., 2014). Soil is not the easiest landscape element to study as the majority of processes taking place are not visible, however recent research efforts and new technologies have vastly improved our capacity to study this cryptic habitat (Campos-Herrera et al., 2013; Häffner et al., 2016; Kada et al., 2008; Lemanceau et al., 2014; Long et al., 2013; Romaní et al., 2006; Vervoort et al., 2012; Zinger et al., 2008). Soil health as "the capacity of soil to function as a living system" (FAO, 2008) has been identified as a key element for sustainable intensification (Bender et al., 2016; FAO, 2015b, 2015a; Lemanceau et al., 2014; Orgiazzi et al., 2016; Reeves et al., 2016; Robinson et al., 2017) as its global degradation is threatening the productivity and stability of agroecosystems (Alyokhin et al., 2019; Amundson et al., 2015; Borrelli et al., 2017; Orgiazzi et al., 2016; Uphoff et al., 2006). Participating in the multifunctionality of agroecosystems, soil organisms can also deliver other key ecosystem services including carbon sequestration and nutrient cycling (Amundson et al., 2015; Bender et al., 2016; FAO, 2015c) and should be considered as a resource in need of adequate management in order to enhance those services (Lavelle et al., 2006), despite those services not being directly valued in existing markets (Huguenin et al., 2006).

Belowground habitat management

In the context of pest regulation through soil health, Altieri, Ponti and Nicholls (2005) called for a "belowground habitat management strategy" and more recently Bender, Wagg and van der Heijden (2016) introduced the concept of soil ecological engineering. As farming practices have long been shown to heavily impact soil biodiversity (Birkhofer et al., 2008a; Gardi et al., 2009; Moore, 1994; Riley et al., 2008; Rusch et al., 2010; Stavi et al., 2016; Tsiafouli et al., 2015; van Diepeningen et al., 2006; Welbaum et al., 2004), we argue that studying how those practices impact on the soil-root pest-natural enemy complex could contribute to the better understanding of pest suppression processes, and help future implementation of conservation biological control strategies, including manipulating the soil as an integral part of the habitat puzzle.

1.4 The cabbage root fly *Delia radicum* and its natural enemies

A root pest hard to suppress, the cabbage root fly

The cabbage root fly, *Delia radicum* L. (Diptera:Anthomyiidae) (Figure 1), is a major pest of wild and cultivated brassica in the Northern Hemisphere (Hughes and Salter, 1959; Coaker, 1969; Finch and Collier, 2000). Adult flies lay eggs at the base of the stem or within 5 cm (Mukerji, 1971) and developing larvae move down in the soil to feed and tunnel in the roots (Finch, 1989) (Figure 2).

Figure 1 The cabbage root fly **Delia radicum © Gedling Conservation Trust**

The main crop damage comes from this feeding, with the larvae occasionally feeding on other parts of the plants, causing considerable damage to the plant as water and nutrient transport are disrupted (Broatch et al., 2006) and also leads to opportunistic infection and secondary rot (Dosdall et al., 2000), which impacts the ability of the plant to survive as well as rendering root vegetables unmarketable (AHDB, 2019, 2015) (Figure 3).

Figure 2 Delia radicum larvae and root damage © Ryan Hudson

Figure 3 Cabbage root fly damage on swede © growveg.co.uk

As adults lay eggs on both cultivated and wild brassica (Ellis et al., 1999; Felkl et al., 2005; Jensen et al., 2002) and can have up to three damaging generations a year in the UK with our current changing climate (Collier et al., 1991), *D. radicum* control is an issue during the entire brassica growing season. Unfortunately, this fly is a formidable foe: overwintering pupae can easily survive -15°C and are capable of supercooling (Kostal et al., 1995), while the adult female can locate a host within 3 km upwind (Finch, 1989; Finch et al., 1982, 1975). The fly was traditionally controlled with chemical pesticides, including

chlorpyrifos as a module drench (Collier et al., 2020; Straub, 1988), however those solutions are now limited after the EU voted against the renewal of its approval in 2019 (European Union, 2019) and after a more general tightening of pesticides legislation in Europe (European Union, 2020). Spinosad, a biopesticide derived from compounds found in the bacterial species *Saccharopolyspora spinose*, is an effective alternative to chlorpyrifos but is substantially more expensive (AHDB, 2013). Cyantraniliprole, a synthetic pesticide belonging to the anthranilic diamide and traded under the name Verimark©, has recently been used successfully by growers (Staples produce, Riviera produce, personal communication) and is, for now, part of the chemical armoury against the cabbage root fly (IRAG, 2019) even though highly toxic to honey bees and moderately toxic to earthworms and most aquatic species (PPDB, 2019).

Common alternative commercial methods of control include physical netting over the crop (AHDB, 2019) (Figure 4), however this method unreliable. Indeed, as flies also thrive on wild brassica weeds, present as volunteer hosts in non-brassica crops, a local *D. radicum* population can become trapped underneath the net after what should have been a suitable crop rotation and lead to complete crop loss (ESG, personal communication). Flies have also been reported landing on top of the net, laying eggs there, with larvae migrating down the plant towards the root. Deer damage and high costs of both net and specialised machinery have also been reported as an issue (ESG and Kettle produce, personal communication). With brassica crops being cultivated on more than 27,000 ha, with 420 000 tons produced and a market value of £245 million in the UK (DEFRA, 2020a), the economic impact of the pest can be considerable, especially considering the standards of the Western market that do not allow for any damage or deformities (DEFRA, 2002).

Figure 4 Brassica netting © Horticulture week

New avenues for control methods

Alternative methods of *D. radicum* control and IPM strategies are currently being researched (Collier et al., 2020; Herbst et al., 2017; Razinger et al., 2017) with some notable success for push pull strategies (Dicks et al., 2016; Eigenbrode et al., 2016; Kergunteuil et al., 2014, 2012) where a cash crop is combined with both repellent intercropped plants and trap plants surrounding the field (Cook et al., 2007). The enhanced knowledge of the chemical ecology of the pest, mainly thanks to the work led by van Dam (Crespo et al., 2012; Papadopoulou et al., 2016; Pierre et al., 2012, 2011; Touw et al., 2019; Tsunoda et al., 2018; van Dam, 2009), Cortesero (Ferry et al., 2009, 2007; Josso et al., 2013; Kergunteuil et al., 2014; Lamy et al., 2020; Neveu et al., 2002; Pierre et al., 2012; Soler et al., 2009; Van Geem et al., 2015) and Hopkins (Hopkins et al., 2009, 1998) is indeed leading to applicable solutions while truly helping to advance our understanding of belowground-aboveground linkages. Other avenues of research for alternative management linked to chemical ecology include trap cropping (Lamy et al., 2018; Rousse et al., 2003) and exploration of wild brassica resistance (Ellis et al., 1999; Felkl et al., 2005; Jensen et al., 2002). Current research also benefits from a solid understanding of the pest behaviour, thanks to the seminal work of Coaker, Finch and Collier (Mowat and Coaker, 1967; Finch and Coaker, 1968; Coaker, 1969; Finch and Skinner, 1975, 2009; Finch and Collier, 1984, 1985; Collier and Finch, 1985; Finch, Collier and Skinner, 1986; Finch, 1990, 1993; Finch and Kienegger, 1997; Finch and

Collier, 2000; Finch, Billiald and Collier, 2003), especially oviposition preference and visual host finding. Parallel to *D. radicum* chemical ecology and behaviour research, a large research effort has been dedicated to habitat and field management in order to enhance the suppression of *Delia* spp. with its numerous natural enemies (Björkman et al., 2010; Dixon et al., 2004; Hummel et al., 2010; Nilsson et al., 2016).

The antagonist community of Delia radicum

Luckily for us, *D. radicum* has a wide range of natural enemies. Eggs, larvae and pupae can all be predated, by either specialist predators including Staphylinid beetles, or by generalist predators such as Carabid beetles, spiders and predatory centipedes, Opiliones and ants (Finch and Collier, 2000; Meyling *et al.*, 2013; Nilsson *et al.*, 2016). Carabid beetles have been the subject of laboratory tests in order to determine their pest predation potential (Finch, 1996; Finch et al., 1992) and studies showed that intermediate size beetles such as *Bembidion tetracolum* Say, *Amara familiaris* Duftschmid, were more effective egg predators than smaller *B. lampros* Herbst, *Trechus quadristriatus* Schrank, or larger *Harpalus rufipes* De Geer and *Pterostichus melanarius* L. (Finch et al., 1992). Finch (1996) found a linear relationship between eggs consumed and Carabid size, between 2.7 mm and 10 mm, while predation from beetles large than 10 mm was highly variable. Andersen *et al.* (1983) found however than *B. lampros* (Figure 5) was the most effective predator and that Staphylinid ate eggs as readily as the Carabid tested.

Figure 5 Bembidion lampros ©Trevor Pendleton http://www.eakringbirds.com

The Staphylinids *Aleochara bilineata* Gyllenhal and *A.bispustulata* L. (Coleoptera: Staphylinidae) both predate the pest but their larvae can also parasitize the fly larvae, at all instar stages (Fournet et al., 2001, 2000; Langlet et al., 1996; Messelink et al., 2004; Read, 1962; Turnock et al., 1995) and the release of *A.bilineata* has even been attempted as an inundation biocontrol technique, with disappointing results (DEFRA, 2002). Parasitism in the field can indeed be very variable with for example values from 0 to 42% reported in England and Wales (Finch et al., 1984).

Figure 6 Aleochara bipustulata ©Trevor Pendleton http://www.eakringbirds.com

D. radicum is also parasitized by wasps, directly laying eggs in the host, including *Tribliographa rapae* Westwood (Hymenoptera: Figitidae) (Hemachandra et al., 2007; Neveu et al., 2000), *Phygadeuon trichops* Thoms. (Hymenoptera: Ichneumonidae) (Finch et al., 1984) and *Gnotus* species (Hymenoptera: Ichneumonidae) (Björkman et al., 2010), with once again very variable impact (Björkman et al., 2010; Nilsson et al., 2016). Negative interactions between parasitoids can occur as pupae parasitized by *T. rapae* can also become hyperparasitized by *Aleochara* spp (Nielsen et al., 2004). As parasitism rate can be highly variable in the field and occurs too late to prevent crop damage from the feeding larvae, this suppression mechanism, highly favoured to fight pests such as aphids, has not often been included in *D. radicum* commercial trials.

The antagonist community of *D. radicum* also include microfauna, such as entomopathogenic nematodes (EPN), entomopathogenic fungi (EPF), bacteria, viruses and

predatory mites present in the soil (Finch and Collier, 2000; Messelink and Slooten, 2004). Laboratory tests have been carried out to test the pathogenicity of several nematode species against *D. radicum*, including *Steinernema feltiae*, *S. carpocapsae* and *Heterorhabditis bacteriophora*, with the majority of isolates being an effective control solution in laboratory or glasshouse conditions (Beck et al., 2014; S. Chen et al., 2003; Shulong Chen et al., 2003; Leger et al., 2009; Willmott et al., 2002). Few studies however report successful results with inoculation in field conditions (Nielsen et al., 2004). Different entomopathogenic fungi can attack both adult flies (Klingen et al., 2000) and larvae, with species such as *Metarhizium anisopliae* (Metschnikoff) Sorokin (Figure 7), *Beauvaria bassiana* (Balsamo) Vuillemin and *Tolypocladium* species having all been successfully tested in laboratory conditions against *D. radicum* larvae (Bruck et al., 2005; Chandler et al., 2005; I. Klingen et al., 2002; Myrand et al., 2015; Vänninen et al., 1999) while *Entomophthora muscae* (Cohn) Fresenius and *Strongwellsea castrans* (Batko &Weiser) have been reported infecting adults in the field (Klingen et al., 2000). Again, few studies report results in field conditions, where so far inoculation of EPF failed to control the pest (Chandler et al., 2005; Herbst et al., 2017). To our knowledge, no studies using viruses against *Delia* have been published while bacterial control using commonly used species *Bacillus thurengiensis* has only attracted a very limited amount of interest, despite the positive results (Havukkala, 1988; Vänninen et al., 1999) and having been found in adult flies in the field (Eilenberg et al., 2000). Also part of the microfauna, the predatory mite *Stratiolaelaps scimitus* (formerly *Hypoaspis miles*) (Acari: Laelapidae) has been reported as an effective biocontrol agent against the fly in greenhouse conditions (Messelink et al., 2004).

Figure 7 Metarhizium species on caterpillar © Nick Sloff

It has to be noted that the vast majority of studies investigating *Delia* spp. suppression with natural enemies focuses on one taxa and only a limited amount of research effort has been spent on investigating the interactions of those natural enemies, notably intraguild predation (Prasad et al., 2004), negative interaction between parasitoid wasp and fungi (Rännbäck et al., 2015) or nematodes and parasitoids (Nielsen et al., 2004), as well as plant growth-fungi-fly interactions (Razinger et al., 2018, 2017). Other more realistic studies including several taxa from the *Delia* antagonist community as well as *Delia* itself focus on habitat and field management and their impacts on the pest-antagonist complex, discussed in the next section.

1.5 Farming practices impacts on the cabbage root fly *Delia radicum* and its natural enemies

Farming practices and field manipulations impacting *Delia* species pest suppression

Field studies investigating management impacts on *Delia radicum* and the closely related species *D. floralis,* as well as their natural enemies, are starting to paint a more complete picture of possible management strategies to reduce the impact of those two pests, using tillage (Dosdall et al., 1998, 1996; Mesmin et al., 2020), intercropping (Björkman et al., 2010; Broatch et al., 2010; Hummel et al., 2010), undersowing (Dixon et al., 2004), flower strips (Nilsson et al., 2012; Nilsson et al., 2016), beetle banks (Prasad et

al., 2006), crop rotation (Dosdall et al., 2012) and by investigating the impacts of contrasting organic and conventional managements (Meyling et al., 2013). Those impacts are reported in more details in Table 1.

Table 1 Summary of publications investigating farming practices impacts on **Delia radicum** *and its natural enemies*

Farming practices	Publications	Impact on *D. radicum*	Impact on natural enemies	Other impacts
Tillage	Mesmin et al., 2020	No significant impact	1- Effect of soil tillage on carabids, spiders and staphylinids did not match the gradient of disturbance induced by tillage treatments. 2- Negative impact of tillage on Carabid that partly overwinter as larvae	-
	Dosdall, Herbut, Cowle, & Micklich, 1996	1- More flies emerged from untilled plots in both years 2- 55-64% reduction in emergence, similar with spring and fall tillage and combination	-	-
Tillage, row spacing, seeding rate	Dosdall, Florence, Conway, & Cowle, 1998	Fly eggs higher in zero tillage	-	1-Higher seeding rate led to lower damage 2-Wider row spacing led to lower damage and higher yield 3-Higher yield in zero tillage even with higher damage
Undersowing with clover	Dixon, Coady, Larson, & Spaner, 2004	1- More eggs in bare plots 2- No difference in pupal numbers	1- Effect of clover was species dependent for Carabid, more *B. lampros* and *A.bifrons* in bare plots, more *P.melanarius* in undersown plots 2- Higher activity density and parasitism of *Aleochara* in bare plots	
Intercropping with clover (*D.floralis*)	Björkman, Hambäck, Hopkins, & Rämert, 2010	1- More eggs in monoculture 2- Reduced pupal numbers in intercropped plots may be more	1- Activity density of predators variable within treatment 2- *Bembidion* spp and *A.bipustulata* more active in monoculture	

		related to lower oviposition than higher pest suppression		
Intercropping with wheat	Hummel, Dosdall, Clayton, Harker, & O'Donovan, 2010	-	1- *A.bilineata* parasitism reduced in intercropped plots in one site.year over four 2- No impact on *T.rapae*	1- damage reduced in intercropped plots
Intercropping with weeds	Broatch, Dosdall, O'Donovan, Harker, & Clayton, 2010	-	1- A.bilineata activity density increased as monocotyledonous weed biomass declined 2- A.bilineata more active in *B.rapa* compared to *B.napus*	
Beetle banks (field and cage experiments)	Prasad & Snyder, 2006	-	1- Predator beetle activity densities increased in fields with beetle banks but not egg predation 2- *P.melanarius* reduced activity densities of smaller beetles, negatively impacting egg predation 3- strength of fly suppression increased in the absence of *P.melanarius*, but not when aphid alternative prey were available	
Flower strips	Nilsson, Rannback, Anderson, & Ramert, 2011	1- No increase of egg density in plots with flower strips 2- Fewer pupae in plots with flowers in one year out of three 3- No effect on *D. radicum* fecundity		
Flower and shelter strips (conservation strips CS)	Nilsson et al., 2016		1- Higher hymenopteran parasitoids activity density in plots with CS 2- No increase in parasitism, higher parasitism in control plots in second year 3- No increase in predation in plots with CS 4- Egg predation correlated with activity of two *Bembidion* species	

			5- Higher activity density of *A.bipustulata* during peak egg laying period in plots with CS	
Crop rotation	Dosdall *et al.*, 2012			1-increase damage after three years of canola 2-decrease in yield with continuous canola production 3- crop sequence effects on *D. radicum* consistent across locations
Organic VS conventional managements	Meyling, Navntoft, Philipsen, Thorup-Kristensen, & Eilenberg, 2013	1- Oviposition generally not reduced in organic plots 2- Higher pupae/egg ratio in conventional plots	1- Activity density of small predators higher in organic plots but predation of eggs not higher 2- Pupae parasitism between 26.5% and 59.5% but no difference between managements	

Tillage has been shown not to have consistent effects on *D. radicum*, as Dosdall *et al.*, (1998) reported higher fly egg numbers in untilled plots, as well as an increased number of emerging flies from untilled plots (Dosdall et al., 1996), whereas Mesmin et al. (2020) reported no significant effect on the pest. Tillage seems to also have contrasting effects on *Delia* natural enemies, as Carabid overwintering as larvae in the soil can be negatively impacted but no consistent negative impact was shown for spiders and Staphylinid (Mesmin et al., 2020). A study in sugar beet systems assessing the impact of strip tillage, in which tillage and seedbed preparation are only carried out in a narrow band, also showed contrasting impacts on Carabid species and a consistent positive impact of strip tillage on Staphylinid, spiders and Opiliones (Wenninger et al., 2020). In winter cereals, reduced or conservation tillage has been shown to increase abundance of ground dwelling arthropods and lead to increased aphid parasitism (Tamburini et al., 2016). Tillage can have contrasting effects on different taxa as physical soil disturbance and distribution of organic matter within the soil profile also matters, both as a resource and also a shelter for numerous soil organisms, including natural enemies (Roger-Estrade et al., 2010). Depending on soil type and crop grown, the formation of beds within the field can be very common in vegetable fields, and reduced tillage might not always be an option for growers.

Undersowing brassica with clover seemed to have positive effects and disrupted oviposition of *D. radicum* (Dixon et al., 2004), most likely through inappropriate/ appropriate landing mechanism, as the fly stands a much greater chance of 'losing' the host plant in a diverse background (Finch and Collier, 2000). The use of different weeds and aromatic plants to disrupt oviposition has been studied by Finch, Billiald and Collier (2003) who showed that fewer eggs were laid on host plants surrounded by fat hen *Chenopodium album* L. (18% total), most eggs were laid on host plants surrounded by common fumitory *Fumaria officinalis* L. (64% total), whereas the five aromatic plants tested made no difference. Depending on the species, leaving weeds in the field can impact the activity of natural enemies, as Broatch *et al.* (2010) showed that a decrease of monodicotyleneous weeds can positively impact *A.bilineata.*

Continuous cropping of brassica has been shown to have a detrimental impact on yield whilst leading to an increase of root damage in oilseed rape systems (Dosdall et al., 2012). Double cropping brassica is however common practice in vegetable growing regions

without clubroot issues (ESG, Riviera produce, Staple produce, pers.comm), depending on land availability and buyers contracts.

Providing shelter, nectar, alternative prey and pollen (SNAP) has been recommended as a strategy to retain and enhance natural enemies populations (Gurr et al., 2012, 2000b). However, research summarised above shows that higher activity density of natural enemies does not always easily translate into enhanced pest regulation (Björkman et al., 2010; Meyling et al., 2013; Nilsson et al., 2016). The reasons for this absence of link are not always clear however the presence of intraguild predators such as *P. melanarius,* as well as the presence of alternative prey items such as aphids can disrupt the target pest suppression (Prasad et al., 2006).

Systemic approach

Similar to a large proportion of research into *Delia* natural enemies, studies investigating the impact of farming practices on *D. radicum* and its natural enemies tend to only focus on one element, which will always only be a part of a larger growing strategy. This informative but reductive approach does not help paint a realistic picture of conservation biological control in field conditions and rarely includes the investigation of other key impacts of the studied strategies, such as soil fertility, plant health or yield. Using a farming system approach, Meyling et al. (2013) compared two organically managed systems to a conventional control to determine their impacts on *D. radicum* suppression, which revealed a higher pupae/egg ratio in conventional plots without showing a reduction of egg numbers in organic plots, whilst also showing an increase in activity density of small predators in organic plots but did not lead to an increased egg predation . As whole farming management strategies, organic and conventional managements will combine farming practices that are typical of or even required for those systems, such as organic or synthetic fertiliser use, crop rotation including legumes, strip cropping, use of synthetic pesticides, or cover crops. Those contrasting managements have been shown to broadly impact soil health and biodiversity (Birkhofer et al., 2008a; Domínguez et al., 2016; Fliessbach et al., 2000; Lohaus et al., 2013; Macfadyen et al., 2009; Scullion et al., 2002; Stockdale et al., 2002) as well as wider agroecosystem biodiversity (Bengtsson et al., 2005; Fuller et al., 2005; Gabriel et al., 2010; Purtauf et al., 2005). Therefore, we then argue that the organic/conventional dichotomy can be used as a starting point to investigate farming

practice impacts on the soil-pest-natural enemies complex, in the hope to highlight the importance of including the soil as habitat in conservation biological control studies.

1.6 Project structure: towards belowground habitat management for conservation biological control of root pests

Project set up

This project was funded by Teagasc, Ireland's Agriculture and Food development authority, through their Walsh fellowship, and in partnership with the Agriculture and Horticulture Development Board (AHDB). The project was registered with the University of Edinburgh, within the School of Biological Sciences and hosted by Scotland's Rural College (SRUC). It was supervised by Prof Bryan Griffiths (SRUC), replaced upon retirement by Dr Alistair Hamilton (SRUC), Dr Andy Evans (SRUC), Dr Michael Gaffney (Teagasc), Prof Richard Hopkins (NRI, University of Greenwich) and with the collaboration of Dr Julia Cooper (Newcastle University) and Prof Tom Little (University of Edinburgh). Whilst based in Edinburgh in SRUC, field work was carried out in Kinsealy (Ireland), Newcastle (UK) and across Great Britain for the commercial soil survey.

Project aim and main research hypothesis

The aim of this project is to assess the impacts of contrasting organic and conventional managements on the soil, the root pest *Delia radicum*, and its natural enemies, in order to highlight potentially beneficial management strategies leading to enhanced root pest regulation.

The main research hypothesis is linked to organic management. If organic management tends to positively impact soil biodiversity and natural enemies' presence, we hypothesize that it can lead to enhanced root pest suppression, both at plot level in experimental rotations and field level in commercial brassica fields. We would expect a higher pest suppression through predation, parasitism and pathogen infection, as well as through stronger plant health thanks to a more biodiverse soil.

Thesis remit

In order to test our main research hypothesis, this project includes some field soil analysis, field pest and natural enemy monitoring, as well as experimentation using field cages and experimentations in controlled conditions using field soils. Field monitoring in experimental fields was carried out to assess the impact of organic and conventional managements on the pest and its entire antagonist community. Subsequently, field soils from those experimental fields were used in controlled conditions experiments in order to assess management impacts on the pest-plant-soil system. Further experimentations using those same soils assessed the potential pest suppression from soil microbial antagonists. Commercial farm soils were also used to study the impact of various organic and conventional managements on the pest-plant-system, as well as the potential pest suppression from soil microbial antagonists. In parallel, soil analysis was carried out in order to quantify the impact of management on soil abiotic and biotic parameters.

Thesis structure

Chapter 2 investigates the impact of conventional and organic management on the soil, the pest, and a wide range of natural enemies including soil dwelling invertebrates. The chapter reports on the results of monitoring that was carried out over two years in an experimental rotation including brassica, in Kinsealy (Ireland). The second year of monitoring also included a field experiment using cages to manipulate pest-antagonists' interactions in field conditions.

Similarly, Chapter 3 investigates the impact of conventional and organic management on the soil, the pest and its natural enemies, this time in a larger experimental rotation including brassica in Nafferton Ecological Farm (Newcastle), once again over two years. This chapter also compares results from both studies sites.

Chapter 4 investigates the impact of organic and conventional management on the potential for microfauna suppression of the pest and plant growth, using Kinsealy and Nafferton soils. Baiting the soil with greater wax moth larvae *Galleria mellonella* L., a common model for entomopathology, was carried out in order to reveal the presence of entomopathogens. In parallel, pest inoculation experiments were carried out in controlled growing conditions using those field soils in order to assess the impact of management on the pest-plant-soil system.

Chapter 5 includes the results of a paired field soil survey, carried out in order to assess the impact of organic and conventional management locally, on commercial farms. This time, no pest or ground-dwelling arthropod monitoring was carried out and only the soil was surveyed. Abiotic and biotic parameters of those soils were determined and the soils subsequently used in the same baiting and inoculation experiments that the experimental field soils in Chapter 4 to determine the managements impact on the pest-plant-soil system as well as the potential pest suppression from soil microbial antagonists.

Chapter 6 presents the general discussion and conclusion of this project.

Summary table of research questions and visual representation of the thesis are included below for clarity.

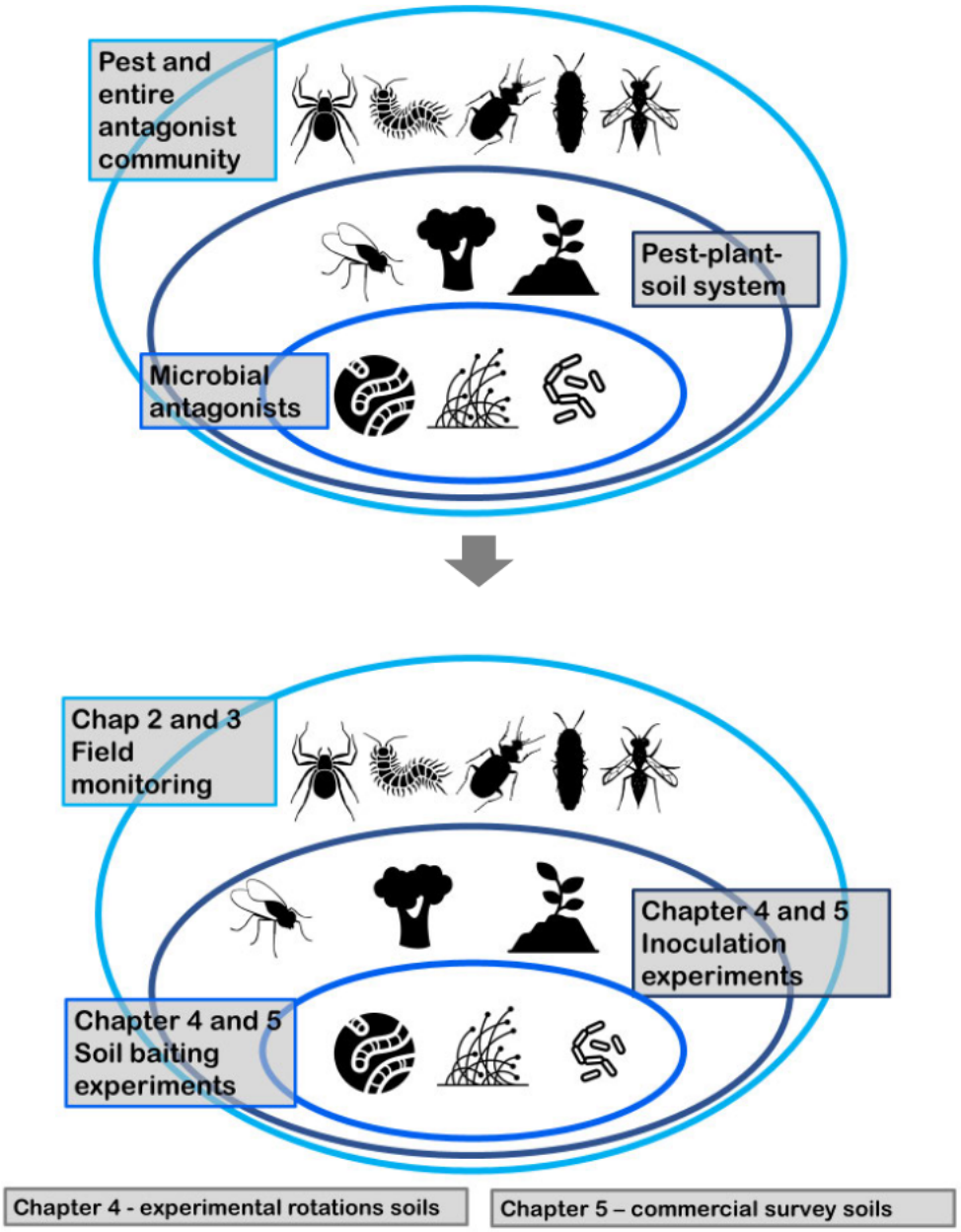


Figure 8 Visual summary of thesis structure - all icons from the Noun project, except Aleochara *author's own*

Table 2 Summary table of the research questions of the four data chapters

Chapter 2 **Farming practices influence cabbage root fly success: lessons learned from monitoring Kinsealy Systems Comparison trial.**	**Chapter 3** **Farming practices influence cabbage root fly survival: lessons learned from monitoring Nafferton Factorial Systems Comparison**	**Chapter 4** **Focus on microbial pest suppression potential and farming practices impacts on the pest-plant system**	**Chapter 5** **Microbial pest suppression potential and farming practices impacts on the pest-plant system in commercial fields.**
1- Does organic management reduce pest survival? 2- Does organic management impact the pest antagonists' community positively at plot level? 3- Can we identify a link between antagonists' activity density and pest suppression within or across samples?	1- Does organic management reduce pest activity and success? 2- Does organic management impact the root pest antagonists' community? 3- Can we identify a link between antagonists' activity density and pest suppression within or across samples? 4- How do Kinsealy and Nafferton sites and practices compare in terms of pest suppression and antagonist community? 5- Can we identify ecological processes and management impacts present on both sites that could help us inform root pest management?	1- Does organic management increase model pest mortality due to entomopathogens compared to conventional management? 2- How does the model pest survival vary over time between systems? 3- How does soil management impact plant growth in controlled conditions? 4- Does organic soil improve bottom up control of inoculated *D. radicum* in controlled conditions?	1- How do conventional and organic managements impact soil parameters? 2- As practices are so varied, is the opposition of organic to conventional still valid? 3- Does local pairing impact soil more than management or inversely? 4- Does soil management impact model pest survival and occurrence of baited pathogens? 5- Does organic management improve pest suppression in controlled conditions?

Chapter 2 Farming practices influence cabbage root fly survival: lessons learned from monitoring Kinsealy Systems Comparison trial.

2.1 Introduction

Agricultural soil can act as a reservoir for a wide range of pest antagonists (Altieri et al., 2003; Alyokhin et al., 2019; Geoff M. Gurr et al., 2004; Klingen et al., 2007). A large body of research has shown the beneficial impacts of low-input and organic managements on soil microorganism communities (Esperschütz et al., 2007; Hartmann et al., 2006; Henneron et al., 2014; Moore, 1994; Orr et al., 2012; Sánchez-Moreno et al., 2009) as well as epigeal antagonist communities (Eyre et al., 2009; Garratt et al., 2011; Holland et al., 2007; Robert L Hummel et al., 2002a; Jacobsen et al., 2019; Letourneau et al., 2001; Pfiffner et al., 2003; Zehnder et al., 2007). Unfortunately, those benefits do not always translate into a reliable improvement in root pest suppression (Björkman et al., 2010; Meyling et al., 2013; Nilsson et al., 2012).

As community ecology is never simple, pest regulation will involve a complex array of ecological processes (Straub et al., 2008; Vandermeer et al., 2019) that can stay somewhat opaque for root pests which take place belowground. Thanks to new tools and renewed soil research effort, those complex linkages between soil and belowground herbivory are slowly being revealed (Alyokhin et al., 2019; Poveda et al., 2006; Rypstra et al., 2005), including the impact of *Delia* spp. herbivory on the soil microbial community (Ourry et al., 2018) and inversely, the impact of non-pathogenic soil organisms on *Delia* spp. (Lachaise et al., 2017; Razinger et al., 2014). Further studies investigating concepts such as intraguild predation or feeding niche complementarity will most likely shed more light on those complex relationships (Jonsson *et al.*, 2008) while new techniques are being developed to help understand those interactions (Birkhofer et al., 2017).

In the meantime, even without understanding all the intricacies of the system at hand, the overall impact of farming practices on conservation biocontrol potential can still be determined. Its monitoring using complementary techniques (Luck et al., 1988) can

inform on top down regulation from the overall antagonist community in parallel with bottom up impact of non-pathogenic soil organisms and wider soil health. This study focusses on the farming practices impacting the soil as a habitat through fertilisation and crop protection, but could not include practices that have clearly been shown to impact conservation biocontrol such as different tillage regimes (Alyokhin et al., 2019; Mesmin et al., 2020; Rusch et al., 2017; Thorbek et al., 2004; Zehnder et al., 2007) or any aboveground habitat elements such as semi-natural habitat, local complexity or habitat manipulation (Bianchi et al., 2006; Brévault et al., 2019; Geoff M. Gurr et al., 2004; Holland et al., 2020, 2017; Douglas A Landis et al., 2000; McHugh et al., 2020; Shields et al., 2019; Tscharntke et al., 2007).

This chapter focusses on the monitoring of a horticulture experimental field, set up to compare the impact of organic and conventional practices at the plot level. If organic farming practices can effectively help attract and maintain a diverse community of pest antagonists and concurrently if the soil antagonist community can have a significant impact on the survival of root pests of field vegetables, we hypothesise that *Delia radicum* survival will be reduced in the organic plots compared to the neighbouring conventional plots, through higher predator activity density and a stronger impact of the microbial antagonist community. In this chapter, predators are considered in more detail whilst the impact of the microfauna will be investigated in more detail in Chapter 4. Due to limited time and resources, parasitoid impact is not included in this study.

As the process of conservation biocontrol is location-specific and its management demands a high level of knowledge of the ecology of the system being considered (Jonsson *et al.*, 2008; Straub, Finke and Snyder, 2008; Begg *et al.*, 2016; Shields *et al.*, 2018; Brévault and Clouvel, 2019), it is crucial to reconcile theoretical knowledge on the pest complex with actual field population data. For that purpose, this study used field monitoring and a cage experiment to identify the main groups of predators co-occurring with the pest and the organic and conventional management impacts on those natural enemies at the plot level, Statistical analysis includes the analysis of the pest and the predators on their own in the different samples, as well as together in correlations across samples, in order to offer some perspective on potential links.

Unlike our Newcastle site, few studies have actually surveyed the cabbage root fly pest complex (including natural enemies and pathogens) in Ireland (see Ryan and Ryan, 1980 in Northern Ireland) so it is hoped that this study can improve the local knowledge of this complex to benefit growers.

The research questions addressed in this chapter are:

- Does organic management reduce pest survival?
- Does organic management impact the pest antagonists' community positively at the plot level?
- Can we identify a link between antagonists' activity density and pest suppression within or across samples?

2.2 Materials and methods

2.2.1 Field site description

Site characteristics

The Teagasc Kinsealy Systems Comparison trial (TKSC, Figure 9) is located in Kinsealy, north of county Dublin, Republic of Ireland (53° 25' N, 6° 10' W). The site is mainly surrounded by semi-managed habitat, urban infrastructure such as Dublin airport, and small pastures. Soil type is loam to clay loam belonging to the grey brown podzolic soil group (altitude, 28 m O.D.; slope, 1°) and is moderately drained (Reilly et al., 2013).

Figure 9 Teagasc Kinsealy Comparison Trial aerial view (Google Maps©, accessed 11-2014)

Set up in spring 2009 for phytochemistry research after ten years of grass set aside, the trial was designed as a factorial strip split plot experimental field, with four replicated blocks containing 128 5.5 m × 3.4 m plots in total (Hernández-Hierro et al., 2012). Organic and conventional treatments are compared on different levels within the Kinsealy site. Each block contained two levels of crop protection treatments (organic protection OP and conventional protection CP, "protection" factor) and two levels of soil fertility treatments (organic soil OS and conventional soil CS, "fertility" factor) as shown on **Error! Reference source not found.**. As buffer zones, 10 m unplanted separation strips were established between crop protection subplots and 5 m unplanted separation strips between fertilization sub-subplots.

Following common commercial rotation practices, broccoli (*Brassica oleracea var. italica*), carrot (*Daucus carota*) and onion (*Allium cepa*) crops were assigned to the plots ("crop" factor), following two parallel sets of rules for the conventional rotation and the organic rotation (Table 3). One variety for each food crop was chosen according to common conventional practices (i.e. variety the most grown in Ireland), the other being chosen with similar characteristics to the first but known to fare reasonably well in organic systems. For broccoli, cv. 'Belstar' and cv. 'Fiesta' were chosen as typical quick cycling varieties commonly grown by Irish growers (65 days to maturity).

Table 3 Rotation rules for Kinsealy site

Organic soil rotation	**Conventional soil rotation**
Ley crop → broccoli→ onion→ carrot Clover as cover crop	Not set pattern Onion crop every 4 years Broccoli crop every 3 years Lettuce as cover crop

Note on rotation use over the years

In October 2013, all plots, representing the full set of crops, were sampled post harvest for soil analysis. In 2014, only broccoli plots were grown with the rest of the plots left fallow, keeping the fully factorial set up. However, in 2015 due to heavy workload and limited resources, only fully organic (OPOS) and fully conventional (CPCS) broccoli plots were grown, leaving the rest of the plots fallow once again.. For the broccoli plots, rotations rules were followed for all years. Caution will be required when considering 2014 and 2015

data as treatments are not entirely equivalent. 2014 soil parameters will be impacted by previous crop. This cannot unfortunately be included in the analysis as previous crop treatment is confounded with soil treatment due to the fixed organic rotation pattern. For instance, 2014 organic fertility broccoli plots all follow a clover cover crop. On the other hand, 2015 soil analysis will not be impacted by other previous vegetable crops as non broccoli plots were left fallow during 2014 growing season.

Local Delia radicum *population*

This site was suitable for *Delia radicum* monitoring as it has been repeatedly heavily infested over the years and pest management targeting this species had to be put in place for other studies using the site (Reilly et al., 2013; Valverde et al., 2014). The neighbouring field has also been used routinely by Teagasc for commercial biopesticides testing against this species (M. Gaffney, personal communication).

A study conducted in 2018 showed that the local *Delia radicum* population belongs to the early phenotype category, as the 148 pupae sampled from the site all emerged within 16 days (Tor J Johnson, personal communication).

The turnip root fly *Delia floralis* commonly co-occurs along with *Delia radicum,* at different ratios (Björkman et al., 2010; Klingen et al., 2002). Both species host range and geographical distribution largely overlap (Alborn et al., 1985; Varis, 1967) and are often studied together (Baur et al., 1996a; Gouinguené et al., 2006; Hofsvang, 1991; Vänninen et al., 1999). The Kinsealy site is no exception but only low numbers (fewer than 10 overall) of *Delia floralis* have been detected in yellow pan traps (2015-2019, M. Gaffney, personal communication).

Management summaries

The experimental rotation plots were managed according to Irish brassica industry standards as advised by the Irish Agriculture and Food Development Authority (Teagasc) extension services. The organic practices used are in compliance with EC1990/92, EC 834/200719/20 and with standards for organic certification set out by the Irish organic certification bodies, with the exception of the unplanted buffer strips. Management plans including dates are summarised below in Table 4 and Table 5.

Greenvale is an organic fertiliser based on pelleted poultry manure and Pro Kali is an organic potassium fertiliser. Bloodmeal is a blood-based organic fertiliser commonly used to increase nitrogen, phosphorus and potassium. Ferramol is an organically approved slug control product containing 1% iron phosphate. Stomp aqua© is a conventionally used broad spectrum herbicide containing 455 gL^{-1} pendimethalin. Gamit© is also an herbicide, containing 360 gL^{-1} clomazone.

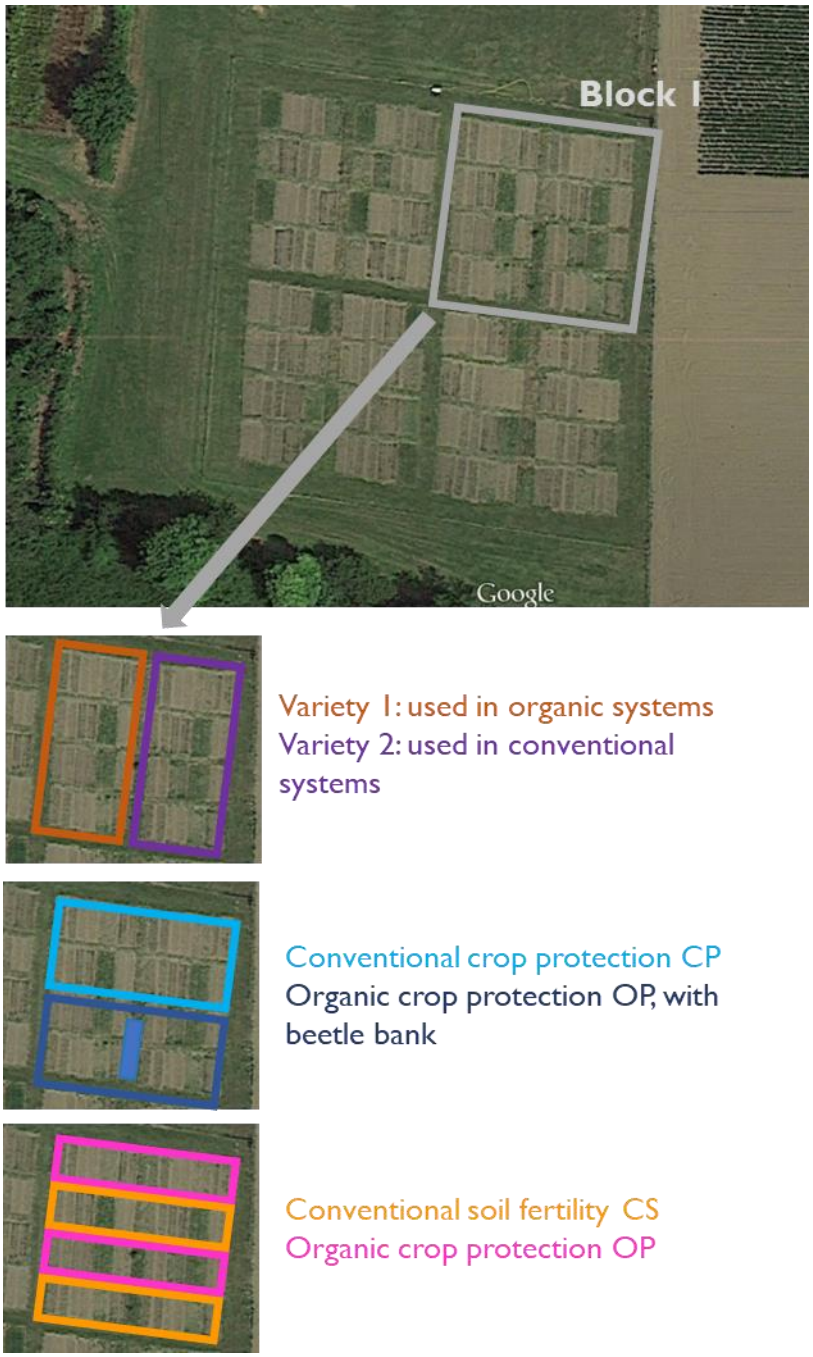


Figure 10 Kinsealy field lay out

Table 4 Management summary for Kinsealy 2014 and 2015

	2014				2015	
	OS	OP	CS	CP	OSOP	CSCP
Sowing plugs in 216 cells trays	27-Mar				26-Feb	
Hardening off	23-Apr				26-Mar	
Fertiliser base treatment and till	29-Apr				10-Apr	
Planted broccoli plugs in plots (45cm between rows)	07-May				13-Apr	
All plots covered with black mesh bird netting (20cm mesh) against pigeons	07-May				14-Apr	
Stomp aqua 2.9l/ha and Gamit 0.25l/ha (2014 only) herbicides		-		07-May	-	15-Apr
Handweeding / hoeing		09-Jun		-	30-Jul, 06-Aug, 11-13-Aug	-
Ferramol (organic approved) slug pellets applied at 7 kg/Ha	19-Jun				21-May	
Fertiliser topdress	26-Jun				14-May	
Amistar fungicide		-		10-Jul	-	
Broccoli harvest	17-Jul				no yield assessment	

OS organic soil CS conventional soil OP organic protection CP conventional protection

OS sowing: in organic compost, OP sowing with untreated seeds, CS sowing in seedling compost, CP sowing with treated seeds

Table 5 Fertility treatments for Kinsealy 2014 and 2015

	Conventional fertility CS	Organic fertility OS
Base treatment per plot	0.73 kg C.A.N (440 $kgha^{-1}$) 0.206 kg Single Super P 16% (125 kgha-1) 0.55 kg Sulphate of K (330$kgha^{-1}$)	1kg Greenvale (600$kgha^{-1}$) 1.56kg Pro Kali (940$kgha^{-1}$) 0.81 kg Bloodmeal (490$kgha^{-1}$)
Top dressing per plot	0.306 kg C.A.N (50 $kgNha^{-1}$)	1.83 kg Greenvale (50 $kgNha^{-1}$)

Management impacts on soil as habitat

This project considers a range of contrasting farming practices under the umbrella of organic and conventional labels, including practices not commonly considered as soil management practices. In order to build our case for including the soil as habitat in conservation biocontrol studies and management plans, showing the significant impacts of managements on soil parameters is an important first step, before focussing on pests and antagonists. Before monitoring the 2014 growing season, the full rotation was sampled for soil analysis in Autumn 2013 as a baseline, in order to characterise the impacts of overall managements on soil parameters, without considering individual management practices listed in Table 4. Significant results of generalised linear models (GLMs) are summarised in Table 6 and full soil analysis protocols can be found in annexes specified below.

Table 6 Summary of soil analysis for 2013 Kinsealy full rotation

Water content (%) (Annex 1)	*Protection*: conventional 19.15±1.64<organic 19.59 ±1.42 GLM F=4.56, df=118, p=0.035 *Fertility:* conventional 19.17± 1.40< organic 19.57 ±1.67 GLM F=3.79, df=118, p=0.054
pH (Annex 2)	*Crop:* broccoli 7.98±0.03<carrot 8.08 ±0.03<onion 8.16±0.02, GLM F=10.72, df= 118 p<0.001
Soil respiration (µg $CO_2.g^{-1}$dry soil) (Annex 3)	*Protection:* conventional 26.46±0.97< organic 28.04±0.84 GLM F=5.20, df=245 p=0.023 *Fertility:* conventional 25.06±0.79< organic 29.45±0. GLM F=12.74, p<0.001 *Crop:* onion 25.95±1.15<carrot 26.57±1.16<broccoli 28.27±1.3198, GLM F=6.10, df=245, p<0.001
Microbial activity (CLPP AWCDt5, Absorbance at 595 µm)	*Fertility:* conventional 1.05±0.03<organic 1.14±0.02, GLM F=7.85, df=118, p=0.006 *Crop:* carrot 1.03±0.04 and onion 1.04±0.03<broccoli 1.26±0.03 GLM F=8.82, df=118, p<0.001
Microbial community (CLPP AUC, PCA) (Annex 4)	*Crop:* PC1 (25.91%) F=6.56, p=0.003; PC3 (7.79%) F=11.52, p<0.001
Nematode abundance (broccoli soil only, g^{-1}dry soil) (Annex 5)	*Fertility:* organic 12.69±1.31<conventional 25.82±4.27 GLM F=10.83, df=24.1, p=0.003
Nematode community (on Broccoli Fiesta soils only, Nematode INdicator Joint Analysis NINJA[1])	Higher abundance of Rhabditidae in fully conventional soils compared to higher abundance in fully organic soils of Cephalobidae points towards a more mature, less disturbed foodweb in the organic soil plots. Ratio of colonisers and persisters nematodes in organic protection soils points toward a greater reliance on the slower fungal decomposition pathway than in conventional protection soils[2].

Overall, 2013 organic soils contained more water, had a higher microbial respiration rate and microbial activity, fewer nematodes but a more stable food web.

The 2013 study conducted by Reilly et al., which focuses on impacts of management on soil microorganisms in Kinsealy, also revealed significantly higher microbial

[1] https://sieriebriennikov.shinyapps.io/ninja/; Sieriebriennikov et al. (2014)

activity and functional diversity in fully organic soils, also using the Community Level Physiological Profile (CLPP) method (Reilly et al., 2013). In this study, culturable bacteria populations were also higher in organic soils and although figures pointed towards a trend of higher fungal and nematode counts under organic management, no statistically significant differences were found (Reilly et al., 2013).

Overall in Kinsealy, fertility practices had an expected positive impact on biotic soil parameters, showing that organic practices used here are mainly beneficial for soil microorganisms and potentially the wider soil food web. As previous crops have also been shown here to have an impact, rotation patterns or lack thereof should also be considered as part of practices impacting the soil but cannot be considered in detail in this project. Crop protection methods should also here be considered as part of practices impacting the soil, as they also had an impact here, even if to a lesser extent than fertility and previous crops.

2.2.2 Field monitoring Teagasc Kinsealy Comparison Trial

Similar to other experimental rotations used for ecological and agronomic studies (Esperschütz et al., 2007; Eyre et al., 2009), the Kinsealy site is comparing organic and conventional practice impacts with some obvious limitations that need to be highlighted before reporting any results.

As we are considering organisms with very different ecology within the rotation, the issue of suitable scale compared to organisms' ecology and range needs to be raised, in order to avoid flawed ecological conclusions (Furlong et al., 2010). Although microorganisms and microfauna such as nematodes might not substantially travel or be transported elsewhere throughout their lifetime, both our pest and the majority of its antagonists will, either by flying or walking. The rotation then becomes more a choice experiment than a true comparison for the mobile pest and antagonists' life stages, with only one overall metapopulation splitting between treatments. Actual management impacts are thus not what is being determined but rather organisms' preferences within this artificial field. However, once reaching a more static life stage, such as larvae or pupae, survival and success can then be assessed.

The second limitation comes from the strip split plot design and the inclusion of beetle banks in the organic protection strips between the two variety sub sections, as well

as the buffer strips between treatments as shown on **Error! Reference source not found.**. Due to the strip structure, in some cases, the conventional broccoli plots were actually closer to the banks than the organic plots. Buffer strips also provide a great network of corridors for antagonists which avoid bare ground commonly found in commercial fields. They also all provide more refuge and overwintering sites compared to cultivated areas, which would benefit the overall antagonist community.

The third limitation is due to the large proportion of semi natural habitat in proximity to the rotation. As Kinsealy is not located within a typical intensive farming landscape and contains a large proportion of wooded areas and private gardens, the local biodiversity will most likely differ in structure and abundance compared to a typical intensively farmed landscape. Regarding the overall abundance, the local wooded landscape could provide more resources and refuges and might encourage higher numbers of antagonists (Bianchi et al., 2006). This would in turn influence the pest regulation services of the rotation. Unfortunately, assessing this overall abundance difference in a quantifiable manner is beyond the scope of this study. Any positive pest regulation results linked to the mesofauna will not be easily transferable to realistic, typical intensive farming landscape.

The only local landscape impact that can somewhat be examined in this project is the potential antagonist spill-over from semi natural habitat depending on proximity to semi natural habitat, by considering sample location within the rotation.

2.2.3 Sampling pest and antagonists

Overall sampling strategy

In order to capture the overall presence of *Delia radicum* in Kinsealy in 2014 and 2015, the first two generations of the fly were sampled, as they are the most damaging for the crop. 3rd partial generation in early Autumn was ignored as broccolis tend to be harvested earlier. Both generation sampling timings were determined thanks to local monitoring as well as *Delia radicum* emergence information available from the AHDB-Syngenta pest bulletin[3].

For pest monitoring, both fly egg and pupa life stages were surveyed, for both first and second generations. For the antagonist community, epigeal antagonists were monitored using traditional

[3] https://www.syngenta.co.uk/ahdb-pest-bulletin

pitfall traps (2014 only) and additionally broccoli root systems were inspected for antagonists during fly pupae extraction. A summary of the overall sampling strategy over time is displayed in Figure 11, with dates in Table 8

Table 8. The levels of replication are described in Table 7 and the plot lay out used for all plots in **Error! Reference source not found.**. Egg sampling was carried out three times during each generation, on the same identified plants, and pitfall traps were used twice per generation. Pupae sampling was carried out once, on plants not sampled for eggs, at the end of each generation.

Table 7 Levels of replication for field monitoring in Kinsealy

	2014	**2015**
Egg samples	4 plants per plot x 4 blocks x 4 combinations of treatments (OPOS, OPCS, CPOS, CPCS) x 3 sampling events per generation n=192 over one generation	4 plants per plot x 4 blocks x 2 combinations of treatments (OPOS, CPCS) x 3 sampling events per generation n=96 over one generation
Pitfall traps	2 traps per plot x 4 blocks x 4 combinations of treatments (OPOS, OPCS, CPOS, CPCS) x 2 sampling events per generation n=96 over one generation	No pitfall traps
Pupae samples	4 plants per plot x 4 blocks x 4 combinations of treatments (OPOS, OPCS, CPOS, CPCS) x 1 sampling event per generation n=64 over one generation	4 plants per plot x 4 blocks x 2 combinations of treatments (OPOS, CPCS) x 1 sampling event per generation n=32 over one generation

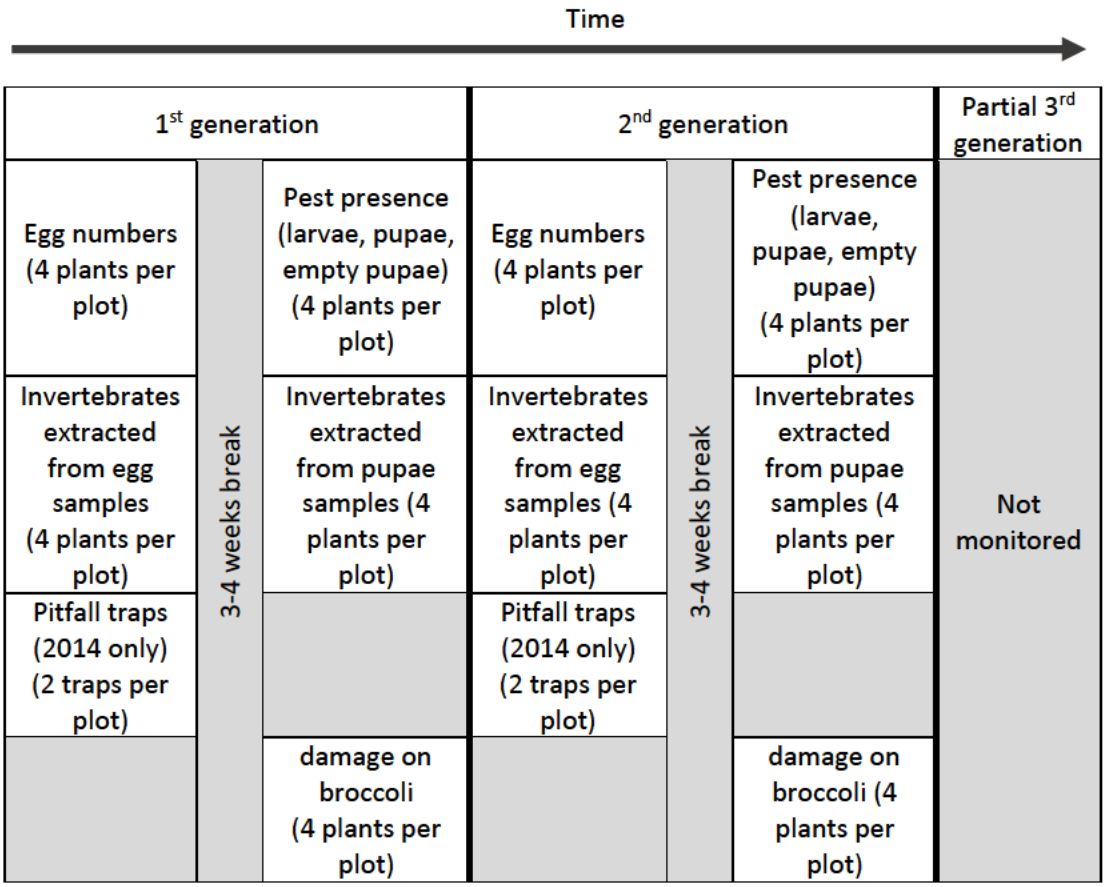


Figure 11 Summary of overall pest and antagonists sampling strategy in Kinsealy

Table 8 Sampling dates for Kinsealy

Year	2014		2015	
Generation	1st	2nd	1st	2nd
Egg sampling	12/05, 19/05, 22/05	14/07, 16/07, 20/07	11/05, 15/05, 19/05	15/07, 17/07, 20/07
Pitfall trap sampling	15/05, 19/05	16/07, 20/07	-	-
Pupae sampling	12/06	15/08	15/06	14/08

The sampling strategy was designed to limit the impact of removal of material as well as avoid sampling the same plants for eggs and pupae. Limited plant numbers led us to carry out egg sampling repeatedly on the same four plants across the two generations. Plants were chosen from the rows inside the plot to avoid the edge effect. All plants were

clearly labelled across both generations. If a plant was missing or too damaged, the neighbouring plant was sampled instead and marked to allow repeated sampling.

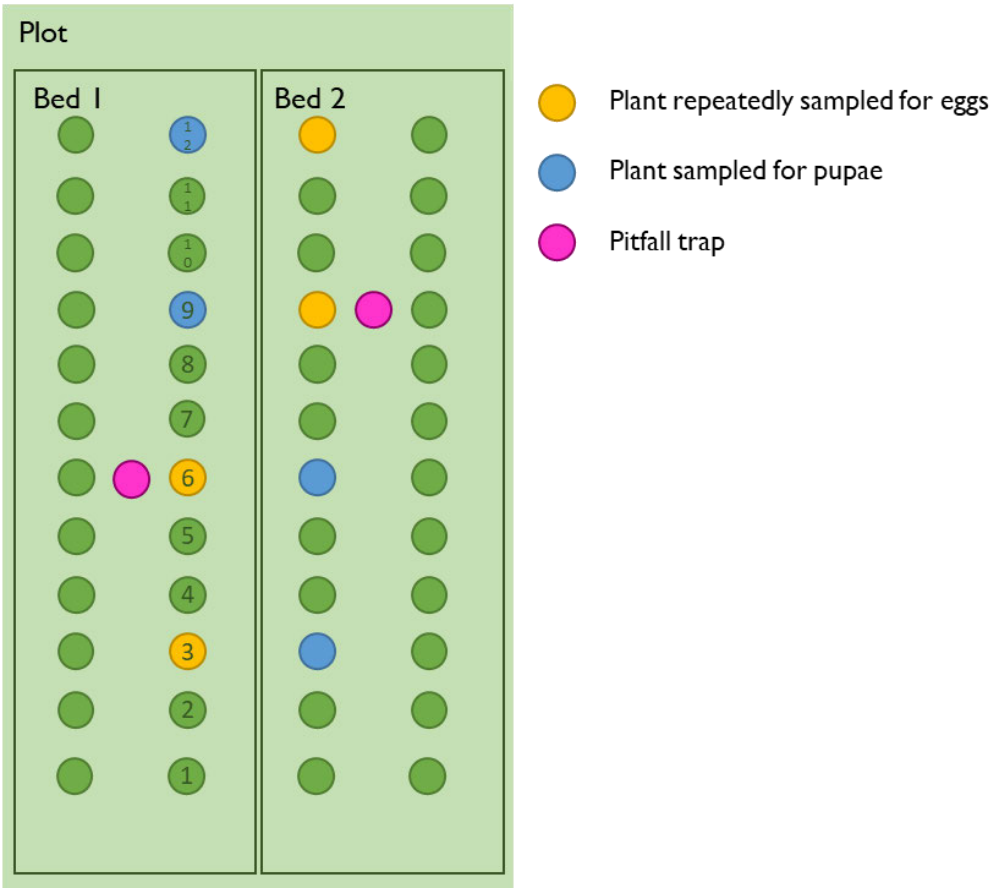


Figure 12 Sampling plan at plot level in Kinsealy used for each plot sampled

Egg sampling and extraction

For each generation, egg numbers were monitored over what was predicted to be peak egg laying activity, over two to three weeks. For both years, egg sampling was carried out in May and July (

Table 8). Soil was removed from around the base of the three labelled broccoli stems with a teaspoon to fill a 40 mL lidded cup. If stones were present, they were inspected for egg presence as flies deposit eggs in clumps on soil blocks or small stones. If eggs were present, they were brushed off the stone in the sampling cup and the stone was discarded. Sampling cups were stored overnight in a 4°C cold store. Egg extraction was done by simple floatation method: the sampling cup was emptied and rinsed in an 800mL beaker filled with cold water, and the soil solution was stirred with a glass rod for 20 seconds. Any floating egg was removed with a paintbrush on a black filter paper to allow

counting and papers were regularly checked under the microscope to confirm that eggs belonged to *D. radicum*. Extra care was taken to brush the insides of the beaker as eggs tend to stick on the sides and stay concealed in the foam. Stirring was done at least three times per sample and a maximum of ten minutes was dedicated to each sample. Any invertebrate found floating in the beaker was also extracted and stored in 70% ethanol for later inspection.

Root system sampling

At least three weeks after egg sampling, we returned to the plots to sample the broccoli root systems. The top part of previously identified plants was cut at ground level and discarded. The entire root ball was dug up with surrounding soil with a 15 cm radius around the stem and 25 cm depth (spade depth), bagged and stored at 4ºC. As soil from the root system was required for further experiments, pupae were not floated but simply extracted with soft stork bill forceps, and placed in a petri dish for counting. Both empty and full pupae were recovered, as well as a few 4th instar larvae still lodged in the root system. Our variable "pupae count" represents the sum of empty and full pupae as well as 4th instar larvae. All invertebrates that could be extracted with the naked eye were also extracted and stored in 70% ethanol. The smallest visible invertebrates in those samples were Staphylinid beetles. No mites could be extracted unfortunately. In 2015 some assistance was provided by our students Michele Quarta and Julia Allen for pupae and predators extraction.

Individual pupal weight was determined to four decimal places using a precision balance (Sartorius model 1872, Sartorius GmbH, Göttingen).The weighing was not carried out for 2nd generation 2015 due to lack of time.

For the first generation of each year, crop damage was assessed after thoroughly washing the lower stem and root system of sampled broccoli. Herbivory marks were assessed on lower stem and tap root using the scoring scale adapted from Hopkins (1994) as shown in Table 9.

Table 9 Stem damage scoring system (Hopkins, 1994)

Score	Criteria
0	undamaged
1	less than 25%of root area damaged
2	25-50% of root area damaged
3	more than 50% of root area damaged
4	more than 75% of root area damaged/severely destroyed

Stem diameter was measured using digital callipers (150 mm DIN 862 ABS/origin, Vogel GmbH, Kevelaer) on the green part of the stem just above the start of roots and textured light brown part as shown on Figure 13. Stem diameter measurement was not carried out for 2nd generation 2015 due to lack of time as plants rotted too quickly.

Figure 13 Example of broccoli stem from Kinsealy prepared for stem measurement

Soil was re-bagged and stored at 4ºC for further experiments

Pitfall trapping

During the egg sampling period, two pitfall traps per plot were laid amongst the rows as shown on **Error! Reference source not found.**. Traps consisted of a 473 ml 8.5 cm top diameter plastic cup dug into the ground with the rim just at ground level, filled with 30 mL of soapy water. Traps were left out for three days. The content of traps was then poured through a 500µm sieve and stored in 70% ethanol. This was done twice per generation, in between egg sampling. Even if plots were covered with bird netting, a few traps were lost to industrious crows. It should be noted that, unlike root system sampling where we actually removed predators where they were present, pitfall trap sampling only

allows for the evaluation of activity density of those invertebrates, as antagonists need to be on the move and walk over the trap to be counted.

Classifying potential antagonists

To classify the potential antagonists, we took inspiration from research very similar to ours carried out by Meyling in Denmark (Meyling et al., 2013) who in turn use Prasad and Snyder methods (Prasad et al., 2006, 2004), mainly focussing on Carabid and Staphylinid beetles and using size range as category. Unlike the Kinsealy site that had never been sampled for beneficial organisms, the Nafferton site was extensively sampled (Eyre et al., 2010, 2007, 2009; M D Eyre et al., 2011) which allowed us to adapt the simple classification to compare both sites (Chap 3). The classification method is described below in Table 10 including non-exhaustive list of species. Taxonomic identification training and support was kindly provided by Dr Lorna Cole (SRUC), Richard Lyszkowski and Ashleigh Whiffin (National Museums Scotland) and the entomology collection team of Oxford Natural History Museum.

Table 10 Classification method for all antagonists extracted from egg samples, root systems and pitfall traps, for Kinsealy and Nafferton, with examples of species

Taxa	Small < 5mm	Medium 6-9mm	Large >10mm
Carabid beetles (ground beetles)	*Bembidion lampros, B. quadrimaculatum, Trechus quadristriatus*	*Anchomenus dorsalis, Agonum muelleri, Pterostichus strenuous, P. diligens, Amara aenea*	*Harpalus rufipes, Pterostichus melanarius, P. madidus, P. niger, Nebria brevicollis, Abax parallelepipedus, Carabus granulatus Leistus* spp
Staphylinid beetles (rove beetles)	*Amisha* spp, *Oxytelus* spp	*Aleochara* spp, *Anotylus* spp, *Aloconota* spp	Fewer than 3 unidentified specimen (*Ocypus* size)
Extra predators/parasitoids	Linyphiid spiders (money spiders), Lycosid spiders (wolf spiders), Dermaptera (earwigs), Opiliones (harvestmen), Coccinellid (ladybirds), beetle larvae, parasitic wasps		

2.2.4 Cage experiment

In order to determine the difference in pest survival with and without the epigeal part of the antagonist community, an cage experiment was performed in 2015 where broccoli plants were grown in the plots within a mesh cage.

At the beginning of the 2015 growing season before planting, two 1x1m locations were identified for exclusion cages within each plot (2 cages x (4 OPOS plots + 4 CPCS plots)) and no broccoli were planted in those locations to keep them pest free (Figure 14). 1x1x0.5m cages were built using timber and Crop Solutions© 0.3 mm aphid net, in order to effectively exclude smaller predators. Initially, those cages were to be dug in 15 cm into the ground, to avoid invertebrates coming in from underneath. However, clay soil texture combined with poor weather conditions caused problems and cages were simply laid on the ground and soil pushed against the structure. One pitfall trap and one yellow sticky trap were set up in the centre of the cage in order to remove as many predators, parasitoids or even emerging flies as possible before the experiment began. As those traps were there only to remove organisms before the experiment, they were not assessed quantitatively. Amongst the potential antagonist organisms, small Staphylinid and Carabid beetles were found on the sticky trap, as well as a number of earwigs and wasp parasitoids. The pitfall trap specimens were very similar to the open field pitfall traps, with fewer Opiliones caught in cages. Two weeks after setting down the cages, broccoli plugs grown following the organic method were planted and both types of traps refreshed. In order to inoculate eggs on field soil and not on plug growing media, plants were left to outgrow their compost plug for another three weeks. Traps were monitored regularly and unfortunately a large number of flies from neighbouring plants emerged within the cages. Traps were inspected for gravid females, but none were found, as no food or flowering weeds were available within the cages for the females to help mature their ovaries. Soil around the stems of the broccoli were checked for egg presence, and only three eggs were found on one plant out of 128 plants and subsequently removed to allow for later artificial egg inoculation.

As the plants in the cages were free from background eggs and flies were caught on sticky traps, inoculation occurred shortly after. Out of four plants, two were inoculated, one with 20 and the other with 30 eggs, and the two others left untouched as control, in order to compare plant growth and potential damage in different soils at the end of the experiment. As the local cabbage root fly culture did not provide enough eggs for the entire

experiment, eggs freshly extracted from the SRUC culture within 24 hrs were mixed with local population eggs. No background hatching test of this mix could be set up as egg numbers were very limited.

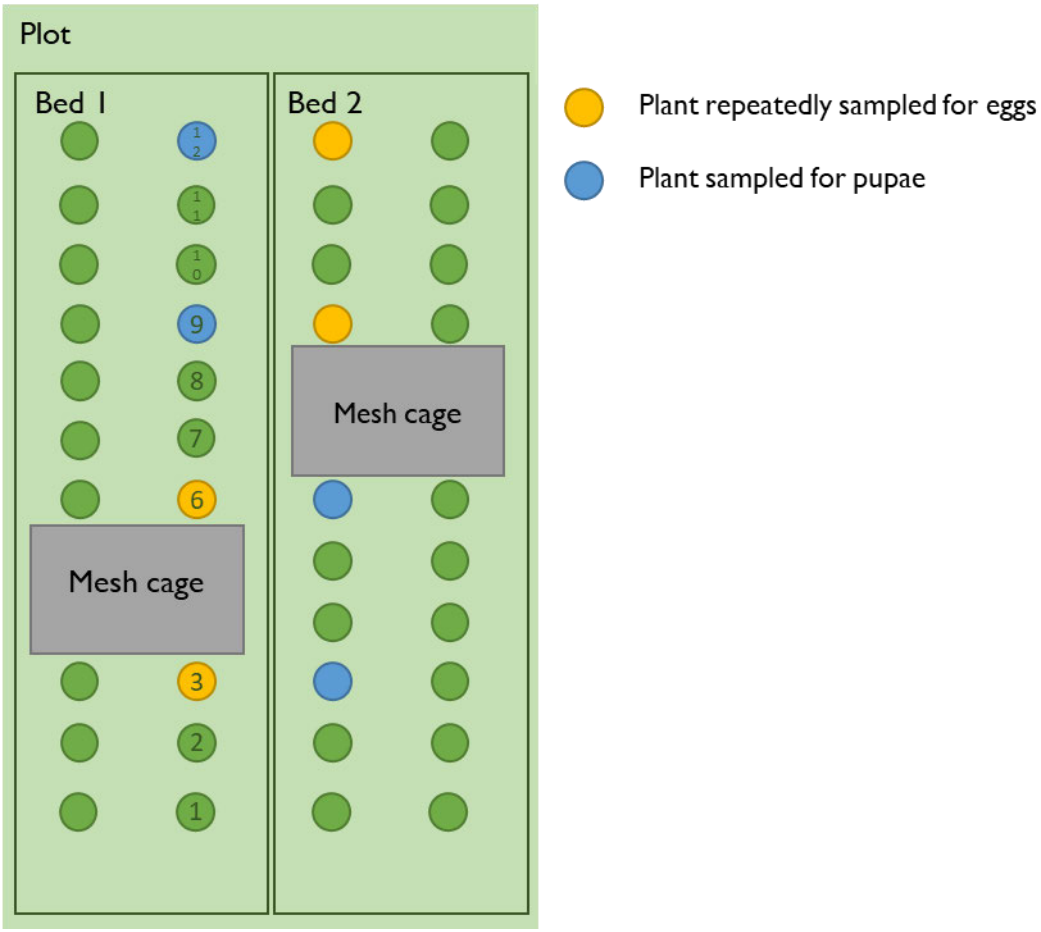


Figure 14 2015 Kinsealy sampling plan including exclusion cages used for each plot sampled

After inoculation, plants and *D. radicum* were left to develop for another month, while traps were monitored regularly. Small Staphylinids were observed coming through the net of some cages and *Bembidion* species were also noticed around broccoli stems. For that reason, the traps were left inside the cage to help continuously remove any emerging predators. All plants and their root systems were sampled at the same time and pupae and invertebrate extraction were carried out in the same way as field monitoring.

The "exclusion" part of the experiment had unfortunately to be redefined as "reduction". Although epigeal antagonists were not completely excluded, gravid female

flies were and egg numbers were controlled on each cage plants. This in turn led us to reconsider this experiment as a cage inoculation experiment, focussing on pest survival in controlled semi field conditions instead of epigeal antagonists' impact.

2.2.5 Statistical analysis

This chapter contains mainly count data, which by nature is discreet and tends to follow a Poisson distribution. Instead of using transformations repeatedly and only being able to conclude on transformed data, raw count data was analysed with Generalised Linear Mixed Model (GLMM), allowing for a Poisson distribution using a Log link function. Fixed models reflect the various treatments considered, such as protection, fertility, soil (protection*fertility), variety, inoculation, and include treatment interactions as well as co-occurring pest or predators count covariates where relevant. Random models reflect the physical layout of treatments of the field studied (block/plot or block/crop protection*fertility/variety for the full factorial analysis) as well as sampling events structure over time for overall analysis (year/generation). Non-count data, such as stem diameter or pupae weights were analysed using General Linear Model after checking the distribution of their error terms, with similar fixed and random model structures than for count data, depending on the variable considered. As cage experiment models differ slightly, they are specified alongside results reported. All means are reported with standard error of the mean (mean±SEM). Analyses were all carried out with Genstat 16 (version 16.1.0.10916, 64 bit edition, VSN International, 2013).

2.3 Results

The first part of the field monitoring result section covers the overall analysis of the different counts, comparing fully organic to fully conventional plots and including both years and both generations. The second part considers results in more detail by also including the factorial element for 2014 sampling and considering year specific effects. The third part covers the cage experiment results.

2.3.1 Overall field monitoring analysis

Fully conventional and fully organic samples were analysed together over the two years and the two generation periods to provide an overall picture of practice impacts. Results are presented by variable measured.

Fly eggs numbers

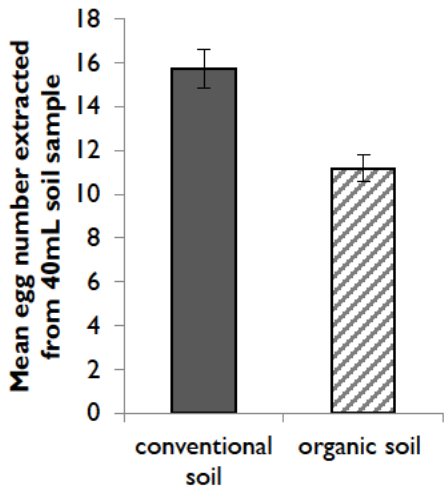


Figure 15 Overall mean egg counts for Kinsealy (±SEM) – fully conventional and fully organic plots only

Over the two years and two generations, organic practices negatively impacted fly egg numbers (conventional= 15.74 ±0.87, organic=11.18±0.59, Soil F= 25.26, df=749.7, p<0.001, Figure 15) with a mean reduction of 29% compared to conventional management. Variety however had no significant overall effect. Count of predators extracted from those egg samples were also included in the model but had no significant impact (Total predators F=1.46, df=763, p=0.227)

Presence of predators in egg samples

No significant differences were found in the presence of predators in egg samples between the two varieties or soil treatments (conventional=0.32±0.03, organic=0.34±0.03, soil F=0.13, df=750.4, p=0.716). Egg numbers from the same sample were also included in the model but had no significant effect (Egg numbers F=1.24, df=719.2, p=0.266). Predators were not present in all 40mL egg samples. In terms of groups, the majority of predators extracted belonged to the medium Staphilinid beetle group (*Aleochara* size) as shown in Table 11.

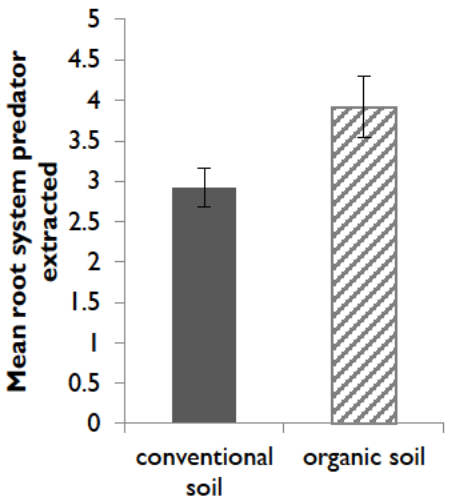
Table 11 Total counts of egg samples predators in Kinsealy, by group

Functional group	Count	Total
Small Carabid	29	50
Medium Carabid	21	
Small Staphylinid	72	285
Medium Staphylinid	213	

Pupal numbers in root systems

Overall analysis of Kinsealy pupal counts revealed no significant impact of soil or variety treatments with similar means for both soils (conventional= 7.94±0.90, organic= 7.41±0.67), contrasting with egg counts. Predator counts from the same samples were also included in the model as a covariate and the GLMM revealed that predators present in the root system were positively related (Total predators F=27.41, df=219, p<0.001).

Root system predators



Overall, organic practices positively impacted predator counts from root systems (conventional=2.91±0.24, organic=3.91±0.38, soil F=, df=3.89, p=0.05, Figure 16) with an increase of 34% compared to conventional plots. Variety did not have a significant impact. Pupae number however had a significant positive impact on predator numbers (pupal numbers F=30.86, df=138.7, p<0.001).

Figure 16 Overall mean counts of root system predators for Kinsealy (±SEM)

As the experimental rotation lies close to semi natural habitat, root system position in the experimental field was also considered in order to check for spill-over and landscape effects. The southern boundary of the rotation was only a few meters away from an established hedge leading to semi natural woodland and the western boundary was around 10 m away from the same type of habitat.

Figure 17 Highlighted plot positions were recoded as "in proximity" to semi-natural habitat

The position of the plant in the field was recoded to identify the plants on the edge on the rotation (closest plant for the southern boundary plots, all plants for the western boundary plots, Figure 17). Including both soil treatments, root systems of plants located on the edge of the rotation had on average 4.17±0.58 predators whereas root systems of plants located within the rotation had an average 3.35±0.25 predators. The difference however was not significant (GLMM Poisson, fixed model soil*landscape, random model year/generation/block, landscape F=1.64, df=91.6, p=0.204) and proximity to semi-managed habitat only reduced pupal numbers in organic plots (GLMM Poisson, fixed model soil*landscape, random model year/generation/block, soil*landscape F=8.15, df=1, p=0.004, effect for organic*yes_proximity = -0.34±0.12, other effects=0).

The vast majority of extracted predators belonged to the medium Staphylinid group, as shown in Table 12.

Table 12 Total counts of root system predators in Kinsealy, by group

Functional group	Count	Total
Small Carabid	90	145
Medium Carabid	55	
Small Staphylinid	160	969
Medium Staphylinid	809	

Pitfall trap catches

Soil and variety treatments had no significant effects on the epigeal predator activity density as measured by pitfall trap sampling (conventional=3.85±0.64,

organic=4.29±0.46, soil F=0.32, df=130.9, p=0.573), contrasting with root systems predator results. Pitfall traps were laid out during the same period as the egg sampling, so egg presence was also included in the model to investigate pest presence impact on epigeal community. Egg presence at plot level was used instead of the plant level, as no pitfall trap could be directly linked to specific egg sample and average eggs per plot per sampling event was used instead of total egg per plot per generation as pitfall traps were laid out between egg sampling events. Average egg counts per plot had a significant positive impact on activity density of epigeal predators (Average egg count F=16.03, df=110.7, p<0.001).

When considering the landscape impact on epigeal predators' activity density, position of the trap was close to having significant impact on the activity density recorded (F=3.55, df=282, p=0.06, edge trap=5.74±0.82, non edge trap=4.71±0.32) with 22% more activity density on the edge of the rotation, whilst neither soil nor variety showed any trend.

Pest and antagonists correlation

In order to help understand how pest and predator samples are linked and contribute to answering our third research question (Can we identify a link between antagonists' activity density and pest suppression), a simple correlation was run including all co-occurring pest and antagonists and is summarised in Table 13. As pupal numbers and root systems predators are the overall results of one generation, total number of eggs per plot per generation as well as total pitfall trap predators per plot per generation were used.

Table 13 Correlation between pest and antagonist counts for both 2014 generations in Kinsealy - Pearson correlation factor (p value), non-significant correlations in grey

Pupae	1	-				
Root system predators	2	0.229 (0.024)	-			
Total eggs per plot	3	0.1862(0.06)	-0.0812	-		
Total pitfall predators	4	0.1923(0.06)	0.0735	0.0241	-	
Total egg predators	5	-0.027	0.0636	0.1963(0.05)	0.2193(0.032)	-
		1	2	3	4	5

Pupal numbers were positively correlated with root system predators within the same sample, and were close to significantly correlated with total egg numbers and total

pitfall predators (p=0.06). Root system predators were not correlated with any other variables. Predators extracted from egg samples were positively correlated with egg numbers, as well as pitfall trap predators.

2.3.2 Focussing on particular sampling periods

Whilst overall analysis provides the bigger picture, looking at specific sampling events can reveal more information on the system dynamics and variability over time. The first year of sampling in Kinsealy included the fully factorial system, comparing organic and conventional soil fertility management (OS, CS) as well as organic and conventional crop protection (OP, CP). Unlike for the overall analysis presented per variable, results are here presented by theme for clarity. In order to assess the importance of the effect reported, the number of times this specific impact is significant is also reported, out of 2 years*2 generations and if there is any change in pattern between different sampling periods (sampling period shortened as “sp”).

Variety impact

Egg counts were significantly smaller at the base of Fiesta broccoli compared to Belstar variety, on two occasions across both years and generations (2/4 sp), with no sampling period with significantly more eggs on Belstar (no switch in pattern). Pupae counts were also significantly reduced in Fiesta plant root systems on one occasion (1/4 sp, no change in pattern), even when there were no differences in egg count on both varieties at the start of the generation. Fiesta plants also tended to have a thinner stem (1/3 sp, no change in pattern), be more damaged (1/2 sp, no change in pattern) even after pest presence was accounted for. Variety impacts are summarised in Table 14.

Table 14 Summary table for variety impacts across years and generations for Kinsealy (mean±SEM)

Variety impact	1st generation 2014	2nd generation 2014	1st generation 2015	2nd generation 2015
Egg numbers	F=5, df=351.4, p=0.026; Fiesta=6.35±0.54 <Belstar=7.56±0.50	Variety NS	Variety NS	F=5.61, df=176.3, p=0.019; Fiesta=11.88±1.88<Belstar=15.57±2.39
Pupal numbers	Variety NS	Variety NS	F=6.96, df=119.9, p=0.009 Fiesta=7.42±0.54 <Belstar=9.36±0.61	Variety NS
Stem diameter	F=5.08, df=80.1, p=0.027 Fiesta=10.21±0.54<Belstar=11.98±0.56	Variety NS	Variety NS	Not measured
Damage score	F=5.05, df=122, p=0.026, Belstar=1.61±0.06<Fiesta=2.32±0.10	Not measured	Variety NS	Not measured

2nd generation build up

When considering pupal numbers, both year and generation had a significant effect (year F=14.69, df=219, p<0.001, year/generation F=27.49, df=219, p<0.001). First generations had similar numbers of pupae over the two years, however 2nd generations samples contained more pupae than 1st generations' ones: in 2014, the 2nd generation pupae count doubled compared to the 1st generation and tripled in 2015 as shown by **Error! Reference source not found.**.

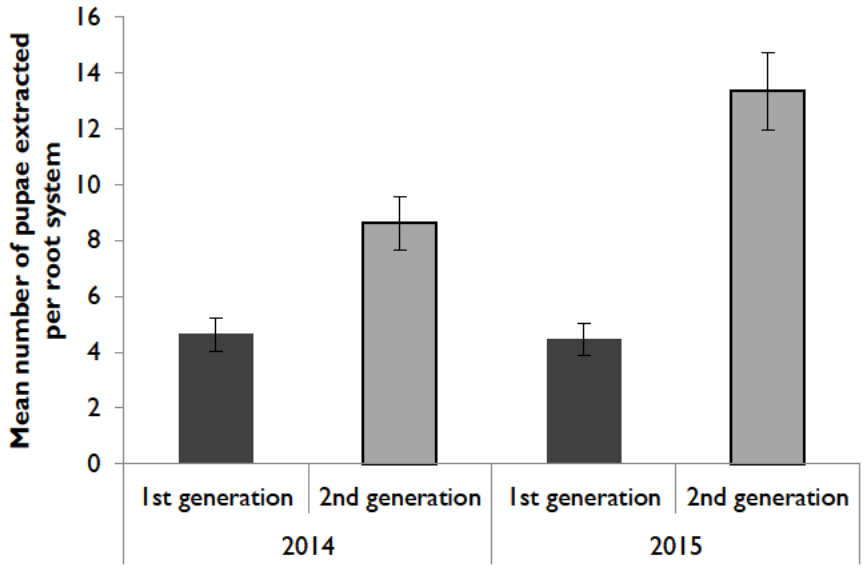


Figure 18 Overall mean pupae counts (±SEM) for both years and generations in Kinsealy – fully conventional and fully organic only

Egg count variations over growing years

Overall egg count was reduced in organic soils, however, if 1st generations of each year are considered independently, a switch in pattern is exposed at the start of the growing season. In 2014, 1st generation egg counts were larger in organically fertilised plots (also larger when considering soil=fertility*protection combined), whereas in 2015, 1st generation egg counts were reduced in fully organic plots (Figure 19 and Table 15).

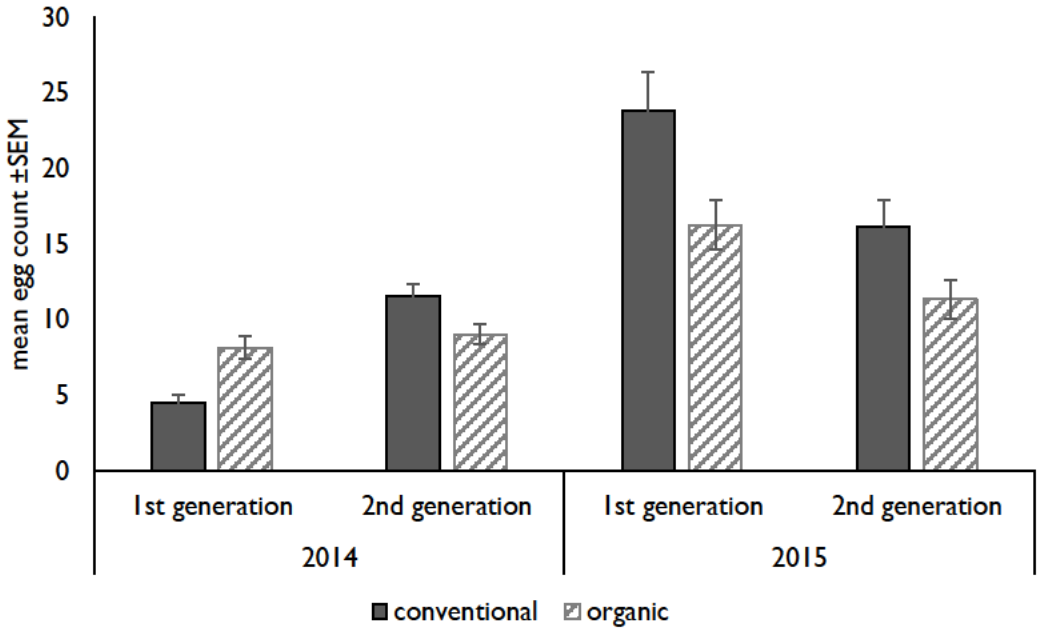


Figure 19 Mean egg count (±SEM) for Kinsealy over both years and generations

Table 15 Summary table for egg counts including 1st generations of both years in Kinsealy

Soil impact (soil=fertility*protection)	1st generation 2014	1st generation 2015
Egg counts	Fertility F= 27.28, df=351.5, p<0.001; conventional fertility=5.06±0.40<organic fertility= 8.84±0.59 (Soil F=16.87, df=178.3, p<0.001; conventional=4.48±0.58< organic=8.15±0.73)	Soil F=6.27, df=41, p=0.016; organic=16.24±1.68< conventional=23.8±2.6

Crop protection impacts: herbicide and weed cover

If we consider 2014 fully factorial systems, contrasting organic and conventional crop protection impacts can be identified. Conventional crop protection included two herbicide sprays (pendimethalin and clomazone) when plugs were transferred to the field at the beginning of May. Organic plots however were mechanically weeded but only at the start of the growing season to reduce competition and this was not necessary once broccoli plants were established. Results are summarised in Table 14. The conventional crop

protection treatment had a transient negative impact on the presence of predators in the egg samples and in the root system, but not on epigeal predator activity density, reduced the stem diameter of sprayed plants as well as pest pupae weight. However, this treatment also led to a reduction in pupae number at the end of the 1st generation as well as a reduction in plant damage. Effects were only present during the 1st generation.

In contrast, the presence of a blanket of chickweed (*Stellaria media*) underneath the organically protected broccoli during the 2nd generation was associated with a reduction in egg numbers but also a reduction in activity density of epigeal predators.

Table 16 Summary table for crop protection effects in 2014 fully factorial system in Kinsealy (mean±SEM)

Crop protection impact	1st generation 2014	2nd generation 2014
Eggs	Protection NS	F=18.8, df=351.1, p<0.001; organic=9.02±0.51 <conventional=12.21±0.62
Egg predators	F=13.67, df=120, p<0.001; conventional=0.37±0.07, organic=0.94±0.15	Protection NS
Pupae	F=6.81, df=120.3, p=0.01; conventional=3.16±0.53 <organic=5.00±0.52	Protection NS
Pupae predators	F=4.79, df=25.5, p=0.038, conventional=3.77±0.39< organic=5.17±0.53	Protection NS
Pitfall traps	Protection NS	F=6.25, df=87.7, p=0.014; organic=6.08±0.58<conventional=8.40±0.95
Pupal weight	F=4.18, df=213.3, p=0.042; conventional=10.24±0.46 <organic=11.66±0.44	Protection NS
Stem diameter	F=41.71, dd=119.4, p<0.001; conventional=12.94±0.48 <organic=13.92±0.26	Protection NS
Damage score	F=11.54, df=122, p<0.001;conventional=1.76±0.08<organic=2.08±0.09	Not measured

2.3.3 Exploring plant-pest system more closely: adding stem diameter, damage score and pupae weights

In order to gain a better understanding of the pest survival, its fitness and its impacts on the crop in field conditions, individual pupae weight, stem diameter and root damage score were recorded when time and resources allowed. Results by variables and by

sampling event are displayed below in Table 17 along with pupal numbers, here included as "pest presence", which collectively represents full pupae, empty pupae as well as 4th instar larvae. Pest presence was also included in the analysis models.

Table 17 Summary table of extra variables measured for plant-pest system in Kinsealy (results in grey for 0.05<p value<0.08, mean±SEM)

	1st generation 2014	2nd generation 2014	1st generation 2015	2nd generation 2015
Pupal numbers (pest presence covariate below)	Protection F=6.81, df=120.3, p=0.01; conventional=3.16±0.53 <organic=5.00±0.52	Variety F=6.96, df=119.9, p=0.009; Fiesta=7.42±0.54<Belstar=9.36±0.61 Total egg numbers F=5.15, df=99.7, p=0.025	All NS	All NS
Pupal weight (mg)	Protection F=4.18, df=213.3, p=0.042; conventional=10.24±0.46<organic=11.66±0.44	Fertility F=3.55, df=210.9, p=0.06; conventional=8.86±0.32<organic=9.81±0.44 Pest presence F=12.93, df=83.2, p<0.001	Soil F=6.58, df=163.2, p=0.011; conventional=8.38±0.24<organic=9.42±0.31 Pest presence F=3.86, df=33.5, p=0.05	Not measured
Stem diameter (mm)	Protection F=41.71, dd=119.4, p<0.001; conventional=12.94±0.48<organic=13.92±0.26 Fertility F=7.62, df=116.3, p=0.007; organic=10.19±0.56<conventional=12.00±0.55 Variety F=5.08, df=80.1, p=0.027; Fiesta=10.21±0.54<Belstar=11.98±0.56	Fertility F=11.39, df=121.1, p<0.001; organic=16.4±0.45<conventional=18.93±0.68	Soil F= 87.21, df=221.8, p<0.001; conventional=6.85±0.12<organic=8.42±0.20	Not measured
Damage score (index)	Protection F=11.54, df=122, p<0.001;conventional=1.76±0.08<organic=2.08±0.09 Fertility F=4.27, df=122, p=0.041; organic=1.68±0.12<conventional=2.04±0.07 Variety F=5.05, df=122, p=0.026; Belstar=1.61±0.06<Fiesta=2.31±0.10 Pest presence F=20.75. df=122, p<0.001 Stem diameter F=15.09, df=122, p<0.001	Not measured	Soil F=7.67, df=48.9, p=0.008; organic=3.32±0.08<conventional=3.58±0.06 Pest presence F=65.65, df=216.3, p<0.001	Not measured

Pupal weights were negatively affected by the conventional crop protection treatment during the 1st generation of 2014 whereas they were positively affected by the organic fertility during the 2nd generation. When considering combined treatments in 2015,

pupae were overall larger in organic soils during the 1st generation. The number of pupae present in the root system also impacted the weight of those pupae (2/3 dp).

Broccoli stem diameter was reduced by conventional crop protection at the beginning of 2014 as mentioned previously as plants were bleached. Fiesta plants were also significantly thinner at the same period compared to Belstar's. During all 2014, organically fertilised broccoli were thinner whereas overall organic management had a positive impact on stems at the start of 2015.

Damage score was mainly impacted by pupae number (pest presence) but was also impacted by protection and fertility treatment at the end of 1st generation of 2014. Damage score was reduced in conventional protected plots as well as organically fertilised plots. When considering overall combined treatment effect during the 1st generation of 2015, damage score was reduced in organic plots.

Correlation plots for both 2014 and 2015 1st generations are shown in Figure 20 and Figure 21 . Firstly, there was a consistent positive correlation between pupal numbers and damage, which even if ecologically rational is still reassuring to find in real field data. Secondly, each year shows contrasting correlations between stem diameter and pest presence. In 2014, there is no significant correlation between stem diameter and damage however there is a significant positive correlation between stem diameter and pest presence. In 2015, there is this time a significant negative correlation between stem and damage but also a significant negative correlation between stem diameter and pest presence, contrasting with 2014. Lastly, pupal weight is only weakly positively correlated with damage in 2014 and not in 2015.

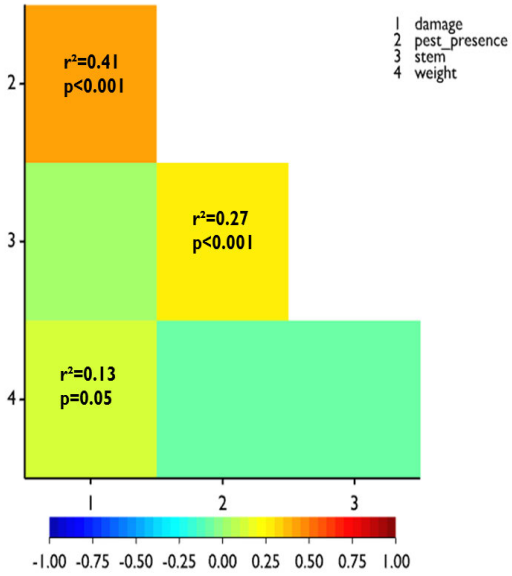


Figure 20 Correlation plot for damage, pest presence (pupal numbers), stem diameter and pupal weight for Kinsealy 1st generation 2014

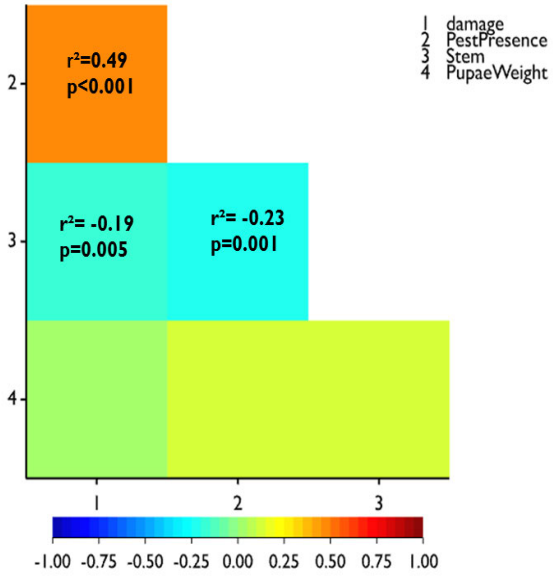
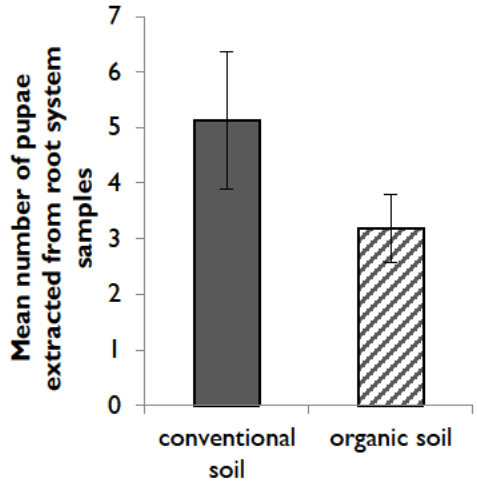


Figure 21 Correlation plot for damage, pest presence (pupal numbers), stem diameter and pupal weight for Kinsealy 1st generation 2015

2.3.4 Cage experiment results

Pupal numbers



Unlike in open field samples, pupal numbers were significantly reduced in organic soil, by 38% (organic=3.18±0.61<conventional =5.12±1.23, GLMM Poisson on inoculated plants, fitted model: soil*variety+predator number, random model: inoculation+block/ plot/cage, F=3.74, df=55, p=0.05, Figure 22). However, contrasting with open field samples, predator numbers did not have a significant impact on pupal numbers.

Figure 22 Mean extracted pupae(±SEM) from both cage experiment soil treatments in Kinsealy

Root system predator numbers

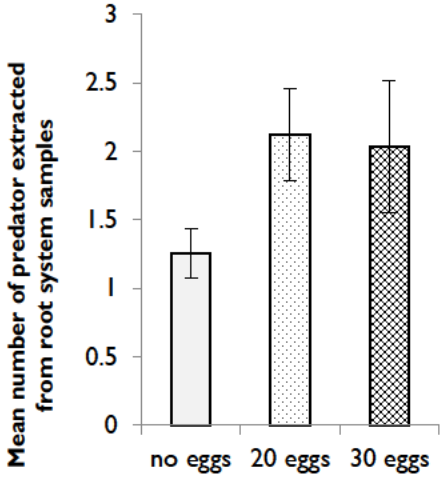


Figure 23 Mean number of predators (±SEM) extracted from cage experiment root systems in Kinsealy, by inoculation level

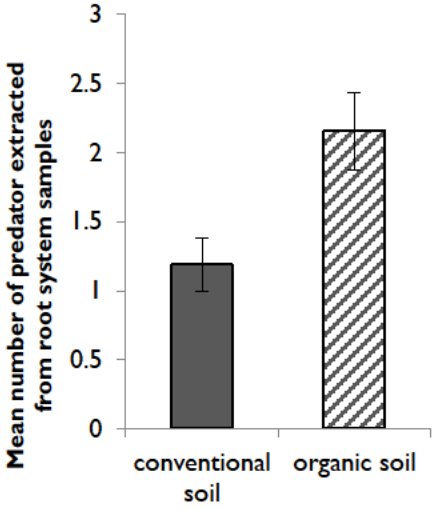


Figure 24 Mean number of predators (±SEM) extracted from cage experiment root systems in Kinsealy, by soil

Predator numbers were significantly impacted by egg presence (inoculation F=3.5, df=119, p=0.033 no egg=1.25±0.18, 20 eggs=2.12±0.34. 30 eggs=2.03±0.48) with significantly more predators extracted from inoculated plant root systems (Figure 23).

Organic soil also had a positive impact (conventional=1.19±0.19, organic=2.16± 0.28, soil F=9.4, df=118.9, p=0.003, Figure 24), but pupal numbers within the same root system did not, unlike in open field samples.

Pupa to egg ratio

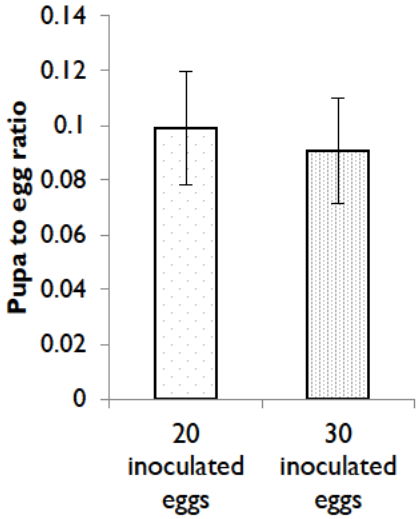


Figure 25 Mean pupa to egg ratio (±SEM) for inoculated plants in cage experiment in Kinsealy, by inoculation level

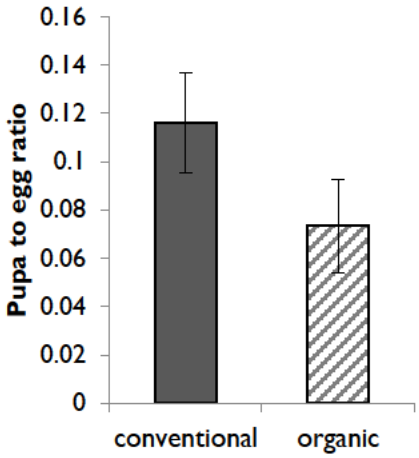


Figure 26 Mean pupa to egg ratio (SEM) for inoculated plants in cage experiment in Kinsealy, by soil

Unlike in open field settings where the actual overall egg numbers being laid are not known as they were only sampled a few times over a generation, the number of eggs inoculated here is known and controlled. Pupa to egg ratio can therefore be used to determine pest success on cage plants, as well as investigate potential impacts of soil, inoculation levels and predator presence. Ratio of pupa/egg was analysed using a GLM on Log(pupa+1/egg) (fitted model: soil+variety+predator number+inoculation, random model: block/plot/cage). Variety and predators had no significant impact. However, inoculation level and soil did. Inoculation level did have an impact with reduced pest success for the higher inoculation level (ratio for 20 eggs=0.09±0.02, ratio for 30 eggs=0.10±0.02, inoculation F=11.33, df=46.6, p=0.002), while organic soil had a negative impact on pest success (conventional=0.11±0.02, organic=0.07±0.01, F=5.58, df=11.4, p=0.037).

Impact of cages on the system

As the exclusion part of the experiment needed to be reconsidered as a reduction of predators instead, comparing belowground figures with the 2015 open field monitoring can give us an indication of the cage impact.

The reduction of predator numbers by the cages and pitfall trap was stronger in conventional plots (Table 16). However, organic plots saw the largest increase in pupal numbers when predators were restricted.

Table 18 Comparison of mean pupae and predators numbers (±SEM) in root systems in cage experiment and open field samples in Kinsealy

	Conventional soil			**Organic soil**		
Field monitoring	2.9±0.23 predators	3.92±0.7 pupae	59.61±4.70 total eggs per plant over 1 generation	3.9±0.38 predators	2.11±0.34 pupae	41.14±3.10 total eggs per plant over 1 generation
Caged experiment	1.18±0.19 predators	5.12±1.23 pupae	20 or 30 eggs	2.16±0.28 predators	3.18±0.62 pupae	20 or 30 eggs
Impact of cages and pitfall traps	-59%	+45%	-58%	-31%	+51%	-39%

2.4 Discussion

2.4.1 Organic management is beneficial but failed to reduce pest survival in open field

At plot scale in the open field, organic management had a negative impact on the start of the fly lifecycle and reduced overall egg numbers. It also led to an increase in the presence of predators in the root systems, both in cage and field conditions, however it only reduced pupal numbers in the cage experiment and not in the open field. Contrasting with root system predators, epigeal predators activity density was not enhanced in organic plots but was almost significantly impacted by the proximity to semi-managed habitat. Second generation pest build up was recorded for both years, with different intensities. Within the same sample, a higher pest presence led to a higher predator presence, thus no suppression links could be easily highlighted. Adding variables to the system and using

correlations helped highlight the variability over time as well as the complexity of the plant-pest-soil system, which should preferably be taken into account in order to produce an informative field monitoring study.

2.4.2 Organic management led to lower egg numbers

Organic management used in Kinsealy led to an overall reduction in egg numbers compared to conventional management, contrasting with the lack of difference found in the similar study of Meyling (2013). Previous studies monitoring *Delia* egg numbers without excluding predators somewhat incorrectly refer to this sampling as oviposition monitoring (Björkman, 2007; Meyling et al., 2013; Nilsson et al., 2011). As egg numbers are a combination of fly oviposition minus egg predation, separating those processes is not straightforward. However, several elements impacting oviposition and predation can be identified in our system.

Firstly, the negative impact of sample removal needs to be considered, both for oviposition and predation. It is known that removal of eggs can have a negative impact on subsequent oviposition as *D. radicum* prefers laying eggs on plant with fly eggs already present (Gouinguené et al., 2006), with roots already damaged by conspecific larvae (Baur et al., 1996). However, we removed eggs across treatments in the same manner whilst our focus is only on assessing differences rather than absolute numbers. We thus assume that the removal of eggs would have had a similar negative impact across treatments and would not help explain the difference found in egg numbers between soil treatments. Similarly, in 2014, we actively removed potential egg predators from the systems with pitfall traps. Once again, as there was no significant difference in the activity density of predators across soil managements, those traps would most likely have had a similar negative impact on overall egg predation and would not contribute to the difference between treatments.

Secondly, even if the detailed assessment of chemical ecology processes is outside the remit of this project, those processes cannot be ignored. A large body of research exists on *D. radicum* chemical ecology, including glucosinolates impacts (Baur et al., 1996a; Ferry et al., 2009; Hopkins et al., 2009; Lamy et al., 2018; Nottingham, 1988; Pierre et al., 2012; Sontowski et al., 2019; Tsunoda et al., 2018; van Dam, 2009; van Dam et al., 2005) and this route seems very promising for the development of sustainable pest control. Aboveground host plant finding and oviposition behaviour have also been extensively researched (Baur et

al., 1996a; Finch et al., 2003; Roessingh et al., 1997) and correlations have long been established between amounts of particular glucosinolates and number of eggs laid (Coaker, 1969; Finch et al., 1977; Nottingham, 1988). Focussing on the contrasting patterns in egg numbers during the 1st generation of both sampled years could potentially give us clues to some of the processes at play. Egg numbers of the 2014 1st generation were significantly higher in organic soil, whereas they were significantly lower in the same generation in 2015. In 2014, due to poor weather, broccoli plugs were late in the ground and egg sampling started only a week later, with pelleted chicken manure still visible on the surface. In contrast, 2015 egg sampling started a month after planting and fertilisation, with no remnant of chicken manure visible. Chicken manure has been shown to emit large quantities of dimethyl sulphide (DMS), dimethyl disulphide (DMDS) along with ammonia (Hobbs et al., 2004). DMDS has been shown to be of ecological importance for the *Delia pest* complex, as it appears to attract its main predators and decrease fly egg laying activity (Ferry et al., 2007) but fails to reduce damage on crop or to reduce retrieved larvae and pupae (Ferris et al., 2009). Recent field studies have shown that DMDS has the potential to reduce oviposition to up to 60% (Ferry et al., 2009; Kergunteuil et al., 2012). Concurrently, an increased amount of DMDS can disturb the foraging activity of the fly predators with a potential reduction in eggs predated (Ferry et al., 2009). Increase in egg numbers in organic plots in 2014 compared to 2015 might not be simply explained by volatiles from chicken manure if they have the potential to reduce oviposition, but it can be hypothesized that those volatiles might have interfered with pest and predator activity early on during 1st generations. In parallel with specific fertilisation impact, overall soil management has been shown to impact glucosinolate content in Kinsealy. Recent phytochemistry work carried out on the Kinsealy site has shown that glucobrassicin and neoglucobrassicin concentrations were significantly higher in the fully organic production system (Valverde *et al*.,2014). Before the discovery of the compound known as “cabbage identification factor” (CIF), glucobrassicin was considered the most powerful stimulant for *D. radicum* (Roessingh *et al.*, 1997). No clear organic management impact can be identified here in terms of reduction of oviposition or stimulation of predation, however, from these elements it can be hypothesized that complex chemical ecology processes impacted egg numbers differently across soil managements.

Egg predation occurs in parallel with oviposition, however the reduced egg numbers in our system's organic plots cannot easily be linked to enhanced predation either. Predators were unexpectedly present in the 40 mL egg samples collected at the base of the stem, highlighting the importance of Aleocharinae as egg predators (Wilde, 1947; Andersen *et al.*, 1983; Fournet *et al.*, 2000; Prasad and Snyder, 2004, 2006b) but their numbers, as recorded in the egg samples, were not significantly impacted by soil management. Those egg sample predators were however positively correlated with egg numbers. Similarly, epigeal predators sampled with pitfall traps during the same period as egg sampling were not significantly impacted by management, but were positively correlated with egg presence in the plot. Those positive correlations point towards the existence of a resource-consumer link where more food attracts more consumers (Hawes et al., 2009), and cannot easily contribute to explaining the reduction in egg numbers in organic plots. Additionally, pupa to egg ratios from our cage experiment were significantly reduced in organic plots, thus potentially pointing towards enhanced predation or microbial suppression in organic plots. Characterising egg predation in open field is commonly assessed using sentinel egg cards, with mixed results (McHugh et al., 2020; Meyling et al., 2013; Prasad et al., 2006, 2004), as previous pest predator studies have highlighted the difficulty in recreating natural dynamics using this method (Lundgren et al., 2011, 2010; Muilenburg et al., 2008). In order to assess predation independently from oviposition, molecular methods such as molecular gut content analysis (Athey et al., 2016; Birkhofer et al., 2017; Furlong, 2014; Harwood et al., 2005; Heimoana et al., 2017; Roubinet et al., 2017; Waldner et al., 2013) would certainly have added depth to this field monitoring.

2.4.3 Organic management led to higher numbers of predators in the root systems but failed to reduce pupal numbers significantly in open field.

Both in open field and cage settings, organic root systems contained a higher number of predators. Whilst the epigeal predator activity density was not impacted by soil management, the belowground habitat in organic plots must have offered more attractive conditions than in conventional plots. As organic management tends to sustain a richer food web (Birkhofer et al., 2008a; Campos-Herrera et al., 2015; Macfadyen et al., 2009; Mäder et al., 2002; Poveda et al., 2006), the presence of more varied food sources could have positively impacted belowground activity of predators, especially through the

detrivorous food web (Birkhofer et al., 2008b). The importance of alternative prey presence in conservation biocontrol has been clearly highlighted over the years (Landis et al., 2000; Prasad and Snyder, 2006a; Harwood et al., 2009; Gurr et al., 2017; Rusch et al., 2017; Gontijo, 2019) however negative impacts have also been identified, in slug control for example where the pest survived and reproduced more quickly in the presence of alternative prey (Symondson et al., 2006) or when beneficial invertebrates are being consumed as much or even more than pests (Tschumi et al., 2018). This negative impact might help explain the lack of reduction in pest pupae in organic root systems containing more predators in open field. This lack of reduction in pupal numbers contrasts with the results of a similar study from Meyling (2013) where the three organic managements consistently led to lower number of pupae over three years compared with the conventional control.

Pupae counts were however reduced by 38% in organic root systems from our cage experiment compared to conventional root systems. Cages only reduced the number of predators and did not exclude them completely but must at least have blocked the free movement of predators on the surface, while the pitfall trap inside the cage contributed to continuously remove predators emerging on the surface. Those disruptions might have reduced intra-guild predation from larger beetles such as *Pterostichus melanarius* present on the soil surface, known to be detrimental to *Delia* suppression (Prasad et al., 2004). Without complete species identification or at least functional group classification (Gagic et al., 2015) or the use of a trait based approach (Gardarin et al., 2018) for the cage pitfall catches and the root system predators, no clear conclusion can be reached regarding the significant reduction of pupal numbers in organic plots in semi field conditions compared to open field conditions.

2.4.4 Co-occurring pest and predators

Since the goal of conservation biocontrol is to reduce pest presence through increased natural enemies activity (Begg et al., 2017; Holland et al., 2020; Jonsson et al., 2008; Rännbäck, 2015; Shields et al., 2019; Snyder, 2019; Torres et al., 2018), the link between pest presence and antagonist activity need to be investigated in the field. As our analysis shows, increased pest presence, sampled as eggs or pupae, led to an increased predator count within the same sample for both life stages. As such, no obvious enhanced pest suppression can be easily identified. If the predators extracted are actually *Delia*

predators, it is not surprising to find a positive link between pest and predators, as within one sampling event more food will attract and sustain more predators in a resource-consumer relationship (Hawes et al., 2009). This significant positive link between pest and enemies goes however against expectations from studies which sample pest and predators at the same time and expected to find a negative correlation, such as Prasad and Snyder (2006), Björkman (2010), and Meyling (2013). In Kinsealy, no negative correlation can be found between pest and predators in our different samples, and pupal numbers were actually positively correlated with pitfall trap activity density, meaning we failed here to identify any pest suppression effect through predation at the end of a generation. In a review of lepidopteran natural regulation studies, Furlong et al. (2010) report that less than half of the 54 field studies reviewed adopted methodologies that actually allowed the assessment of the impact of predators on the studied pest. In order to evaluate impacts of natural enemies adequately, Luck et al. (1988) suggested the use of a combination of complementary techniques, including cages and removal of natural enemies, which we attempted but failed to implement correctly. The exploration of belowground trophic interactions will always benefit from using more than one approach (Lundgren et al., 2011), which may be lacking in this study. This topic will be further developed in the next chapter which analysed both experimental sites together.

2.4.5 Contrasting predator sampling techniques

Epigeal predator activity density, as measured with pitfall traps, was not significantly enhanced in organic plots, contrasting with the predators present in the root systems. Pest presence in the form of egg numbers had a significant impact on this activity density, contrasting with other *Delia* studies using sentinel eggs and pitfall traps (Björkman et al., 2010; Meyling et al., 2013; Prasad et al., 2006). Proximity to semi managed habitat almost had a significant positive impact on this activity density, in line with previous results and prescriptions (Bartual et al., 2019; Holland et al., 2020; Mchugh et al., 2020). Even when commonly used in agricultural research, pitfall traps limitations have long been identified. Focussing on Carabidae, Luff (1975) studied the impact of different trap features on the trap ability to catch Coleoptera. Small traps (2.5cm) were shown to be the only traps suitable to catch small beetles such as *B. lampros*, present in our system, and those small beetles escaped the most from large traps, similar to the ones we used (Luff, 1975). Bias for larger body size of commonly used pitfall traps has also been identified by Hancock and

Legg (2012) whilst Halsall and Wratten (1988) did not find size to have an impact when trapping Carabid beetles. Unlike with our root systems where we removed individuals where they were physically present, individuals have to actively walk over the trap and not escape to actually be counted, introducing the notion of trappability of a predator (Melbourne, 1999). One study in an arable field environment reveals that in general, abundance of Carabid and Lycosid were overestimated by pitfalls, while Staphylinid and Linyphiid were underestimated (Lang, 2000).

Further comparison of root system and pitfall traps communities will be discussed in the next chapter when comparing sites, where those biases will be further highlighted. Here we can start however by pointing out the potential inadequacies of pitfall traps to study the natural enemies of a root pest. To our knowledge, no other studies focussing on *Delia* natural enemies in experimental plots considered enemies co-occurring with pupae in the brassica root systems and only relied on pitfall traps (Björkman et al., 2010; Meyling et al., 2013; Nilsson, 2011). In our study in Kinsealy, whilst pitfall trap activity density was indeed positively correlated to numbers of predators extracted from egg samples, it was not correlated with root system predator numbers. At the same time, whilst pitfall trap activity density was closely impacted by surrounding landscape, root predators were not. Inversely, root system predators were impacted by management, while epigeal predator activity density was not. Those differences would point towards contrasting impacts of management on epigeal predator's activity density, compared to belowground predators, and clearly highlight the need for the use of complementary sampling techniques when studying natural enemies (Luck et al., 1988; Lundgren et al., 2011). Sampling only epigeal natural enemies of a root pest seemed somewhat inadequate, as molecular techniques have pointed towards the stark contrast between root pest predation carried out by the epigeal community compared to the belowground community extracted from soil columns (Lundgren et al., 2011).

2.5 Conclusion

In Kinsealy, organic management led to a significant reduction in *Delia radicum* egg numbers in both open field and semi field conditions, but only reduced pupal numbers in cage conditions. Whilst it enhanced the presence of predators in the root system, organic management did not have a significant impact on the epigeal predator activity density, as measured with pitfall traps. Complementary sampling techniques highlighted the

limitations of using only pitfall traps and the use of correlations helped give a clearer picture of the limitations of field sampling strategies. Indeed, the main link found between pest and predators was a resource-consumer link and no obvious pest suppression link could be determined when considering our different samples. Adding variables to our pest-plant-system gave us a glimpse of a more complete picture, however spending more resources on natural enemies' identification would have been a wiser choice. Not specifically impacting the soil as habitat, other elements in the system such as variety and landscape also had an impact on the pest and its natural enemy community, highlighting the difficulty of identifying the adequate elements to include in such a study, in order to inform farmers who, unlike researchers, do not have the option of simplifying their systems.

Chapter 3 Farming practices influence cabbage root fly survival: lessons learned from monitoring Nafferton Factorial Systems Comparison

3.1 Introduction

This chapter contains the field monitoring results from our second long term factorial experimental trial on Nafferton Farm, comparing the impact of organic and conventional management on *Delia radicum* and its antagonist community. The Nafferton Factorial Systems Comparison trial represents another example of commonly used organic and conventional practices, set this time in a more typical farming landscape and following an arable crop rotation.

Soil as a habitat does not seem to be clearly recognized as part of the puzzle in recent conservation biocontrol reviews (Begg et al., 2017; Shields et al., 2019) even when impact of tillage and fertilisation on pest regulation are discussed elsewhere (Hummel et al., 2002a; Thorbek and Bilde, 2004; Klingen and Haukeland, 2006; Roger-Estrade et al., 2010; Rusch et al., 2017; Alyokhin et al. , 2019). Soil as an integral part of the landscape has seldom been mentioned since the work of Altieri and Nicholls (Altieri, 1999; Altieri and Nicholls, 2003) with research focussing mainly on aboveground landscape and non-crop habitat manipulation (Gontijo, 2018; Jonsson et al., 2008; Rebek et al., 2005; Tscharntke et al., 2007; Woltz et al., 2012) or more recently on agroecosystem redesign (Pissonnier et al., 2019; Pretty, 2018). Improving our understanding of soil pest and their antagonists' preferences at plot level might help make the case for including the soil habitat in more conservation biocontrol studies and help integrate elements of soil-focussed research relevant to pest regulation such as food web manipulation (Lundgren et al., 2011; Macfadyen et al., 2009) and aboveground-belowground interactions (Birkhofer et al., 2008b, 2008a; Blouin et al., 2005; de Vries et al., 2013; Van der Putten et al., 2001; Van Der Putten et al., 2009; Wardle et al., 2004).

Replicated plot field trials create very artificial agroecosystems and cannot reflect commercial settings or integrate the adequate ecological scale (Furlong et al., 2010). However, comparisons carried out across independent populations and sites, investigating management impacts in different systems, also tend to suffer from methodological problems and do not often lead to clear quantitative conclusions, as they have to take into considerations the vast numbers of factors affecting each individual field (Hole et al., 2005; Letourneau et al., 2008). With those constraints in mind, monitoring management impacts on pest and antagonists in those conditions can help us reveal impacts at a "plot scale" (term used in Eyre *et al.*, 2009; Eyre and Leifert, 2011), removing field and site variability and allowing us to focus on the aboveground-belowground interactions fundamental to ecosystem functioning (Bardgett et al., 2014; Birkhofer et al., 2008a; Kabouw et al., 2011; Van der Putten et al., 2001) . Especially as we can find differences in predators preferences at a very small scale, such as in the root systems of the 5.5 m x3.4 m plots in Kinsealy where organic management increased root predator activity, or in the studies from Eyre et al. on beneficial invertebrates studies in Nafferton (Eyre et al., 2013, 2012, 2009; Eyre et al., 2011). If we focus on understanding how those antagonist populations split between the surface habitat and belowground, as well as between plots, and investigate potential links between pest and antagonist activities, we can perhaps help answer some questions regarding conservation biocontrol and habitat manipulation, and why it sometimes fails (Tscharntke et al., 2016).

The Nafferton site monitoring benefits from a larger body of previous research carried out by soil scientists as well as ecologists. Unlike in Kinsealy where antagonist sampling was carried out without any prior knowledge of the local community, we were able to start with a detailed understanding of the local management impacts on beneficial organisms thanks to the work of Eyre *et al.* (2009, 2012, 2013), especially including some information on local Carabid beetle preferences as well as management impacts or lack thereof on soil parameters (Cooper et al., 2011; Orr et al., 2012, 2011). In turn, those studies led us to expect significant difference in antagonists' activity between managements, even with limited significant impacts of management on the soil itself.

Similar to Kinsealy, the Nafferton site was monitored both for pest and antagonists' activity over a two year period using complementary sampling techniques in order to gain the required knowledge about the local managed system and management impacts, and

assess their location specific conservation biocontrol potential (Begg et al., 2017; Jonsson et al., 2008; Shields et al., 2019; Straub et al., 2008).

This chapter's research questions are aligned with the previous chapter, with *two additional questions*:

- Does organic management reduce pest activity and success?
- Does organic management impact the root pest antagonists' community?
- Can we identify a link between antagonists' activity density and pest suppression?
- *How do Kinsealy and Nafferton sites and practices compare in terms of pest suppression and antagonist community?*
- *Can we identify ecological processes and management impacts present on both sites that could help us inform root pest management?*

In order to compare sites, work carried out in Nafferton had to be as similar as possible to the one carried out in Kinsealy. To answer the first additional question, a similar sampling strategy and the same methods were used as in the previous site in order to produce a similar dataset, to compare the impacts of two sets of practices as well as identifying site specific elements. For the second question, having produced similar data would allow overall analysis of both sites together, aiming to identify dynamics and processes present in both sites but perhaps not obvious with individual analysis.

3.2 Materials and methods

3.2.1 Field site description

Site characteristics

The Nafferton Factorial Systems Comparison (NFSC) trial is located on the University of Newcastle's Nafferton Experimental Farm, Northumberland, U.K (54:59:09 N; 1: 43:56 W) (Figure 27).

Figure 27 Nafferton Factorial Systems Comparison site aerial view (Google Maps©, accessed 11-2014)

The trial site is located on the organic side of the farm and is mainly surrounded by organic or intensively managed farmland. Soil type is sandy loam of the Stagnogley type. Set up in 2001, the field first saw a conversion period after being conventionally managed, using untreated grass/red clover ley until 2003, according to Soil Association standards and cultivation was staggered until 2004 to allow for different rotation patterns (Palmer et al., 2013). Unlike the Kinsealy site which was set up for phytochemistry research, the aim of this field trial was to study the effects of low-input and organic food production systems on crop and food quality as well as safety (Orr et al., 2011). The trial was designed as a strip-strip plot experimental field, with four replicated blocks, over 6 ha. The effects of crop rotation, crop protection, and fertility management can all be assessed, as well as their interactions. Crops are grown following two sets of rules, either following a diverse rotation, rich in legume and potato/vegetable crops as recommended by organic farming principles (ORG rotation) or a non-diverse rotation, dominated by cereal crops typical for conventional systems (CON rotation). Each rotation main plot is divided into two crop protection subplots following either conventional farming practice (Red Tractor Farm Assured Combinable Crops standard) or organic crop protection standards (Soil Association organic farming standards). In turn each of the crop protection subplots is divided into two fertility management sub-subplots (final plot size 12 m x 24 m) following either

conventional farming practice (Red Tractor Farm Assured Combinable Crops standard) or organic farming standards (Soil Association organic farming standards). Crop protection subplots and fertilization subsubplots are randomized, and 10 m unplanted separation strips are established between crop protection subplots and 5 m unplanted separation strips between fertilization sub-subplots (Cooper et al., 2011). Lay out can be found in **Error! Reference source not found.**. Where vegetables were to be grown, plots were further divided into two strips, with one for potatoes and one for cabbages (Figure 29). Cabbage plots were further split in two parts, with one section of the plot netted with mesh (Capatex 1.3 mmx1.3 mm) to protect the plants against *D. radicum.* Only the un-netted part of the plot was sampled as shown on Figure 29.

Cabbages were only included in the organic rotation, thus conventional rotation could not be included in this study and the effect of previous crop was not assessed. Unlike in Kinsealy, only one variety of cabbage ("Amazon") was grown over the three years sampled. Table 19 shows the previous crops present on cabbage plots sampled over the years.

Table 19 Previous crops present before cabbages

Year	Previous crop	Previous crop
2014 cabbages	Potatoes	Beans
2015 cabbages	Potatoes	Beans
2016 cabbages	Grass/clover ley	Winter wheat

Note on rotation use over the years

All 64 cabbage plots were sampled in Autumn 2014 to assess the presence of *D. radicum* before deciding on sampling strategy for 2015 and 2016. In 2015, the fully factorial system was sampled for the 1st fly entire generation as well as for the 2nd generation egg sampling. However, due to both sites overlapping that year, only the 32 fully conventional and fully organic plots were sampled for 2nd generation pupae of 2015. Again in 2016, only fully conventional and fully organic cabbage plots were sampled.

Local Delia radicum *population*

2014 Autumn sampling revealed the presence of a local *D. radicum* population so the site was deemed suitable for our study. No other information is available on the local fly population but as the great majority of our reared pupae emerged within 16 days in the

laboratory, it can be assumed that the local population belongs to the early phenotype category, as with Kinsealy. No information is available on local occurrence of *D. floralis*.

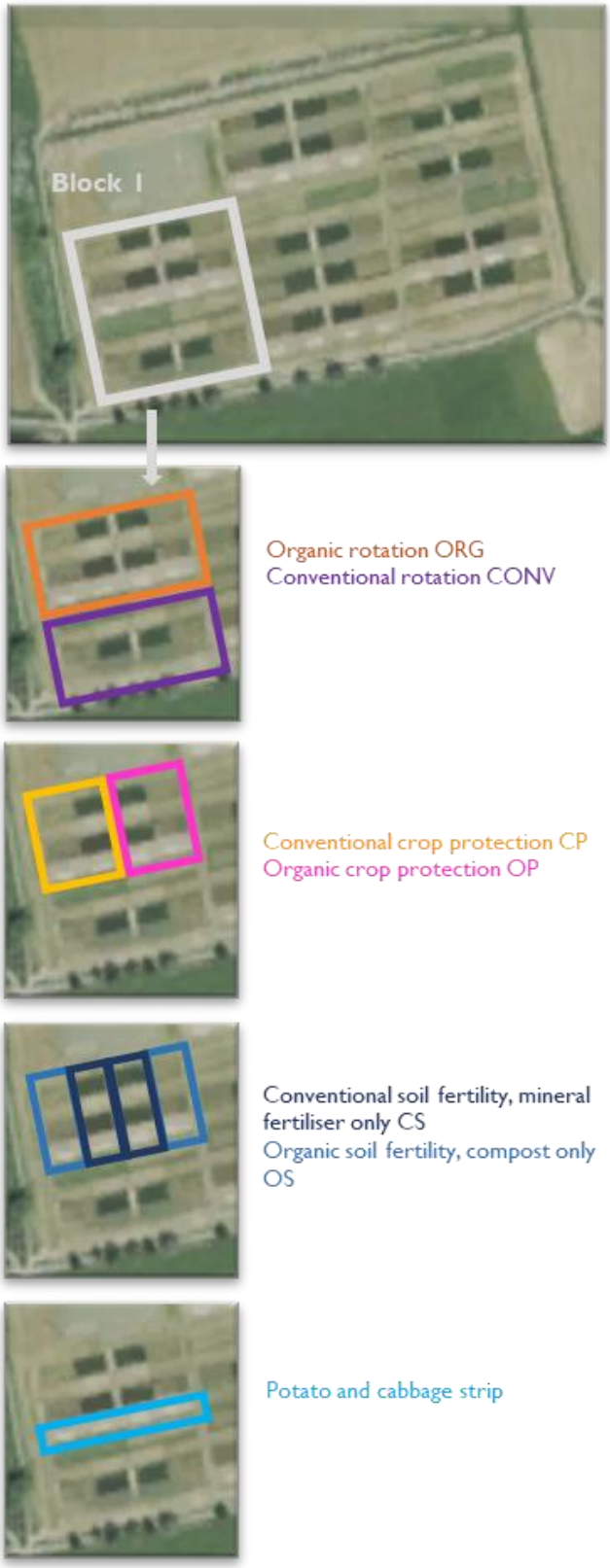


Figure 28 Nafferton field layout for block 1

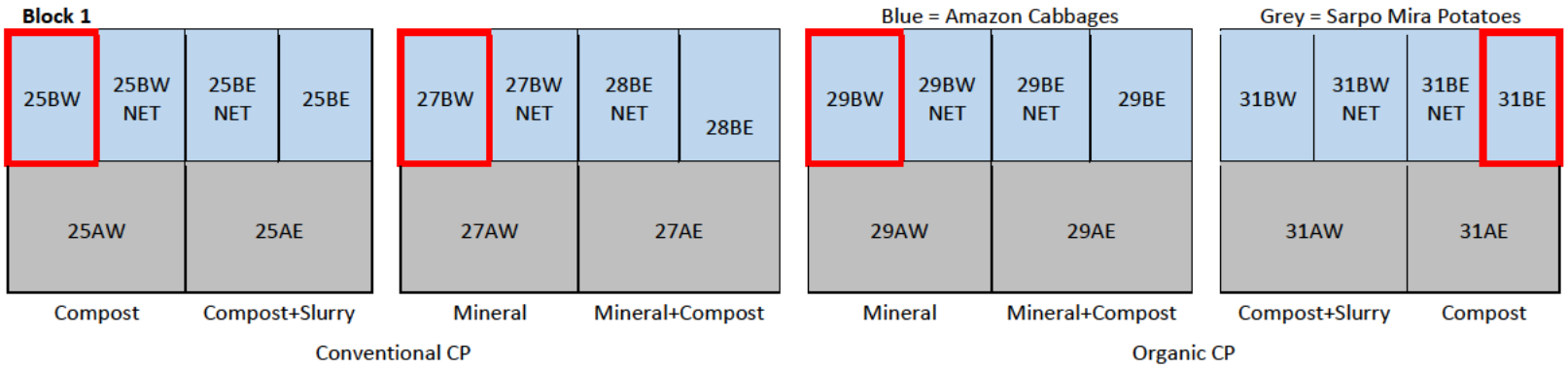


Figure 29 Example of fully factorial cabbage plots "B" for Nafferton organic rotation including neighbouring potato plots "A", with the two crop protection subplots "W" and "E"- sampled part of the plot in red (6x12 m)

Management summaries

Organic plots were fertilised with dairy manure adjusted for 250 kg total N ha^{-1} late March/early April of each year. The only crop protection used in the organic system are the nets for cabbages mentioned above. Conventional management is summarised below in Table 20.

Table 20 Conventional management summary for Nafferton cabbages plots, 2015 and 2016

2015	2016	Treatments
28-May	24-May	planted cabbage plugs and P&K fertiliser applied (2015 150kgP/ha:330kgK/ha, 2016 100kgP/ha:150kgK/ha
04-Jun	03-Jun	applied Metazachlor herbicide 1.5L/ha
12-Jun	09-Jun	applied Nitram fertiliser (2015 50kgN/ha, 2016 100kgN/ha)
16-Jun	20-Jun	applied Lentagran herbicide 2kg/ha
19-Jun	20-Jun	applied Alto Elite Fungicide 1L/ha
19-Jun	20-Jun	applied Chlorpyrifos insecticide 1L/ha
24-Jun	20-Jun	applied Nitram fertiliser (2015 50kgN/ha, 2016 50kgN/ha)
24-Jun	08-Jul	applied Laser herbicide 1L/ha
-	08-Jul	applied Amistar fungicide 1L/ha
24-Jun	08-Jul	applied Cropsray adjuvant 1.25L/ha
22-Jul	-	applied Alto Elite Fungicide 1L/ha
22-Jul	20-Jul	applied Chlorpyrifos insecticide 1L/ha

For clarity, a summary of sites management is included below in Table 21.

Table 21 Site comparison summary

Kinsealy	Nafferton
• **Two varieties of broccoli**	• One variety of cabbage
• **No replicated rotation, previous crop and soil treatment confounded**	• No replicated rotation, cabbages only grown in organic rotation
• **Field vegetables only**	• Include cereals and potatoes
• **Using pelleted chicken manure and calcified seaweed**	• Using farmyard manure
• **No *Delia* specific pesticide used**	• Use of chlorpyrifos drench against *D. radicum* in conventional plots
• **Smaller plots 5.5×3.4 m**	• Larger plots 12x24m
• **Use of bird netting**	• Soil type: Sandy loam
	• Set up in 2001
• **Soil type: loam to clay loam**	• Located in typical farmland landscape
• **Set up in 2009**	
• **Surrounded by semi-managed habitats, gardens and woodlands**	

3.2.2 Management impacts on soil as habitat

No overall soil sampling and analysis was carried out in Nafferton for this study. Previous research on the Nafferton site however has been able to evaluate the impacts of the different managements on different part of the agroecosystem.

Soil bacterial community structure and activity were mainly affected by the crop rotation as in Orr et al. (2011) whilst the effect of fertility management and crop protection were not consistent across the years studied. Although activity of soil organisms increased under organic fertility management and pH was significantly reduced in conventional fertility plots (Orr et al.,2012), the main factors explaining variations were temporal and seasonal effects. The same study also reports an unexpected lack of difference between soil organic carbon and total N between organic and conventional management (Orr et al., 2012). Unpublished data from a 2016 MSc thesis by S. Gilliland analysing cabbage plots soils, summarised in Table 22, also failed to detect a significant difference between total carbon, total nitrogen, potassium, and soil organic matter between treatments. The same project however showed that phosphorus was lower in organic plots and iron tended to be higher in conventionally fertilised plots ($p= 0.06$). $N0_3$ N and NH_4 N concentrations were strongly increased in the conventionally fertilised soils however potentially mineralisable

nitrogen was higher in organically fertilised soils. Similar to Orr *et al.* 2012, soil basal respiration was enhanced under organic fertilisation.

Table 22 Soil analysis for 2016 cabbage plots (6% significance in grey) - source S. Gilliland MSc thesis, unpublished data, Sept 2016.

		Fertility management	Crop protection
Macronutrients	P	org 21.16±7.86 <conv 27.73±8.48 (p=0.013)	NS
	Ca, Mg	NS	NS
Micronutrients	Fe	org 422.68±45.35 <conv 434.10±40.91(p=0.059)	NS
	Mn, Ni, Cu, Zn, I, Se	NS	NS
Nitrogen	$N0_3$ N	org 43.46±22.89<conv 104.57±33.06 (p=0.007)	NS
	NH_4 N	org 1.37±3.97<conv 30.38±14.19 (p=0.018)	NS
	Potentially mineralisable NH_4 N	conv 19.04±20.41<org 47.93±19.29 (p=0.034)	NS
	Total N %	NS	NS
Carbon	Total C %	NS	NS
	SOM %	NS	NS
Soil basal respiration		conv 2.04±0.36<org 2.45±0.18 (p=0.023)	NS
pH		NS	NS

Organic fertility benefits were somewhat less clear here than in Kinsealy's soils (Chap 2, Table 4), with only an increase in potentially mineralisable nitrogen and soil basal respiration.

In terms of management impacts on productivity, lower yields were reported in organically fertilised plots for potatoes (Palmer et al., 2013) and wheat (Cooper et al., 2011) with inter-year variability having a significant effect.

Even if crop rotation seems to have a large impact on soil parameters as identified in those previous studies, we will not be able to include pre-crop in the present study as cabbages are only part of the organic rotation schedule. Significant in the majority of those studies, inter-year variability and sampling timing effect will also be expected here.

3.2.3 Management impacts on beneficial invertebrates

Unlike in Kinsealy, beneficial invertebrates were previously researched in Nafferton, including potential antagonists of *D. radicum.* Eyre et. al (2009) investigated the effect of the different managements on 11 groups of beneficial invertebrates, mainly predators and parasites, over two growing seasons. This study showed that crop type had the strongest effect, followed by fertility type. Crop protection surprisingly perhaps only had a limited impact on beneficial invertebrates. Most differences were found in cereals and grass/clover crops, with fewer significant results in vegetables and beans. Carabid beetles and Lycosid spiders were more active in the organically fertilised plots whereas Staphylinid beetles, Lyniphiid spiders and Braconidae wasps were more active in conventionally fertilised plots. Two additional studies focussing on Carabid beetles (Eyre et al., 2013, 2012) also reported the main effect of crop type, with reduced activity in vegetable and spring barley plots compared to beans and winter barley plots. Those studies included species level data and size grouping, thus providing precious information on which beetles to expect in our cabbage plots as well as their levels of activity. The 2012 study includes information on vegetable plot activity density of the 20 most abundant Carabid species. Table 23 reports activity from the most abundant species found in vegetables plot, as well as crop type where those species are most abundant. This includes previously identified *D. radicum* predators such as *Bembidion lampros* and *Trechus quadristriatus* (Mitchell, 1963) but also the intraguild predator *Pterostichus melanarius* (Prasad et al., 2006, 2004).

Table 23 Most abundant (n>10) Carabid species means (±se) in Nafferton vegetables plots over the 2005-2008 period - (Eyre* et al. *2012)

Species	Present study group category	Total over 4 years in vegetable plots	Most active in
Bembidion aeneum	Carabid small	14±1.3	31±3.2 in winter barley
Bembidion lampros	Carabid small	93±6.6	vegetables
Bembidion tetracolum	Carabid small	59±4.8	72±7.0 in beans
Nebria brevicollis	Carabid large	58±4.2	126±11.4 in beans
Pterostichus melanarius	Carabid large	95±7.6	164±27.3 in beans
Trechus quadristriatus	Carabid small	38±2.5	56±7.1 in beans, 56±4.4 in winter barley

Eyre, Luff and Leifert (2013) investigated the effect of field boundary type, productivity and disturbance on Carabid beetles over a five year period. The major variation in species distribution was a product of crop type and boundary type. No information was included on vegetable plots in particular, however short herbaceous boundaries were included (Table 24). As this field trial contains a large number of short herbaceous strips, as buffer between treatments, information on Carabid species preferences and level of activity in those can help us understand the local dynamics and dispersion of potential *D. radicum* predators, outside the cabbage plots.

Table 24 Most abundant (n>20) Carabid species totals in Nafferton field boundaries over the 2005-2008 period - (Eyre* et al.*2013).

Species	Present study group category	Total over 5 years in short herbaceous boundaries	Most active in
Anchomenus dorsalis	Carabid medium	23	Short herbaceous boundaries
Bembidion aeneum	Carabid small	20	Short herbaceous boundaries
Bembidion lampros	Carabid small	223	Short herbaceous boundaries
Bembidion tetracolum	Carabid small	22	Short herbaceous boundaries
Loricera pilicornis	Carabid medium	63	Short herbaceous boundaries
Nebria brevicollis	Carabid large	81	Short herbaceous boundaries
Pterostichus melanarius	Carabid large	293	Short herbaceous boundaries
Pterostichus niger	Carabid large	41	Short herbaceous boundaries
Pterostichus strenuus	Carabid medium	26	Hedge (42) and woodland (42) boundaries
Trechus quadristriatus	Carabid small	64	Short herbaceous boundaries

3.2.4 Comparing practices at plot level

The species information in Table 23 and Table 24 provides additional background information on the Nafferton trial as overall habitat and the preferences of known *D. radicum* antagonist species, as well as some intraguild predators. Similar to Kinsealy, this trial can only compare management effects at interdependent plot level, and as such counts and activity densities must be regarded as preference rather than true comparison. Once again, the local metapopulation of pest and antagonists will split in different ways according to resource presence and habitat preference. Compared to Kinsealy where only broccoli crops were grown in the studied years, with the rest of the rotation staying fallow, our Nafferton field monitoring will be impacted by the presence of other crops within the trial. Luckily for us, thanks to the Eyre studies (Eyre *et al.*, 2009; Eyre *et al.*, 2012; Eyre, Luff and Leifert, 2013) we benefit from background information on species preferences as well as impacts of management.

Two main points need highlighting. During the monitored years 2015-2016, no field beans were grown in Nafferton. Table 23 shows a large overlap between the most numerous species of Carabid found in vegetable plots and beans plots. Spring bean plots could not act as a sink during our monitoring years, as this crop was not grown. Potato plots were neighbouring cabbages during both years without any grass strip separation, as cabbages and potatoes are always grown on neighbouring half plots in the Nafferton rotation (see Figure 29) but no specific beneficial invertebrates information is available regarding those plots. Other neighbouring crops, displayed in Table 25, might indeed act as sinks during our sampling years as the activity density of predators tended to be lower in vegetables, apart from *B. lampros*. In Eyre et al. (2009), Staphylinid beetles were most active in spring barley and the least active in vegetables, whereas Carabid beetles were most active in wheat and barley, along with beans. Spiders, categorised as “extra predators” in our study, had also different preferences with Linyphiid significantly more active in grass/clover during the two years, whereas Lycosid were most active in grass/clover one year and beans the next. Apart from *B. lampros*, it can be expected that antagonist activity will be negatively impacted by neighbouring crop, as well as the short herbaceous strips present between blocks, subplots and sub-subplots, however this effect will not be quantified.

Table 25 Neighbouring crops in Nafferton over the two years of field monitoring

	2015	2016
Neighbouring crops of cabbage plots	Winter wheat, spring barley, grass/clover, potatoes	Winter wheat, spring barley, grass/clover, potatoes, spelt/rye

The second point worth highlighting is the size of the Nafferton plot compared to the size of differences expected. In Kinsealy with 5.5 m x 3.4 m plot size, we failed to detect any management impact on epigeal predator activity, also with only one year of pitfall trap data, and could only show that egg presence in the plots had an impact on those predators' activity. In Nafferton, with plots more than double the size, both Eyre *et al.* (2009) and Eyre *et al.* (2012) were able to detect significant differences in activity at plot level. Even with only one year of data, compared to two years (Eyre et al., 2009) and four years (Eyre et al., 2012), we might hope to detect differences in epigeal predators activity density in Nafferton.

3.2.5 Sampling pest and antagonists

Overall sampling strategy

Similar to Kinsealy, the first two generations of *D. radicum* were sampled in Nafferton, over the two years 2015 and 2016. The timing of sampling for both generations was determined using the same AHDB-Syngenta pest bulletin tool [4], as well as local monitoring from Nafferton farm staff. Both eggs and pupae were sampled for each generation once again, and epigeal predators' activity-density was monitored with pitfall traps in 2016. Due to lack of time, stem damage was not scored in Nafferton. Overall sampling strategy is displayed in Figure 30, levels of replication in Table 26 and sampling dates in Table 27.

[4] www.syngenta.co.uk/ahdb-pest-bulletin

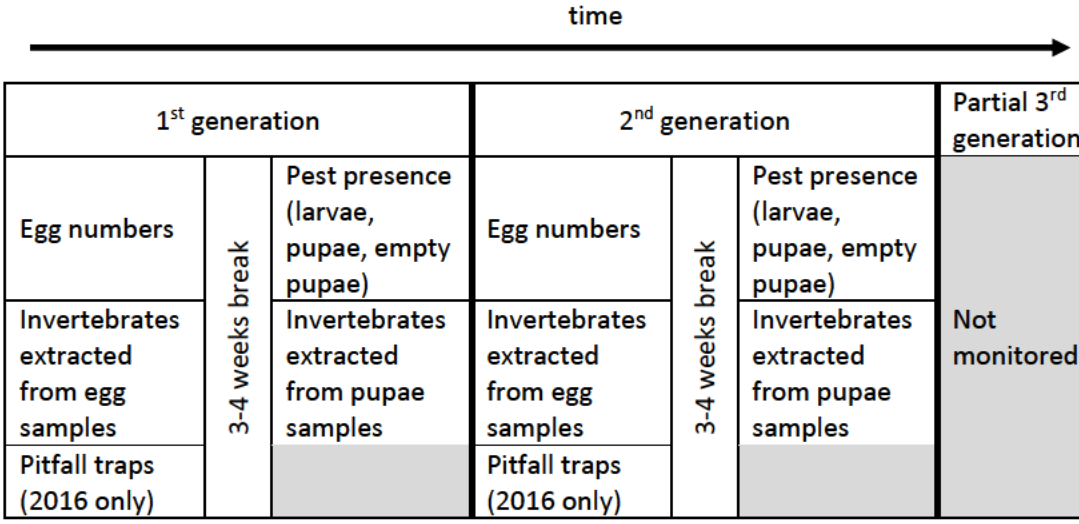


Figure 30 Summary of overall pest and antagonists sampling strategy in Nafferton

To limit sampling interference, plants were clearly labelled to allow for repeat egg sampling at the start of the generation and avoid sampling those plants to assess damage at the end of the generation. All sampled plants were chosen from the middle bed of each plot and from the inside rows of this bed to avoid edge effects, equally dispersed along the length of the subplot. Unlike in Kinsealy where all plants were numbered within the plot to repeat exactly the same sampling across these very small plots, Nafferton's larger plots offered more flexibility, so plants were not numbered and counted, and sampled plants were chosen at what was estimated to be four equidistant points along the middle bed (Figure 31). Due to strong hare damage in 2016, some plants were replaced in block 1 after 1st egg sampling and new plants were labelled for the remaining egg sampling.

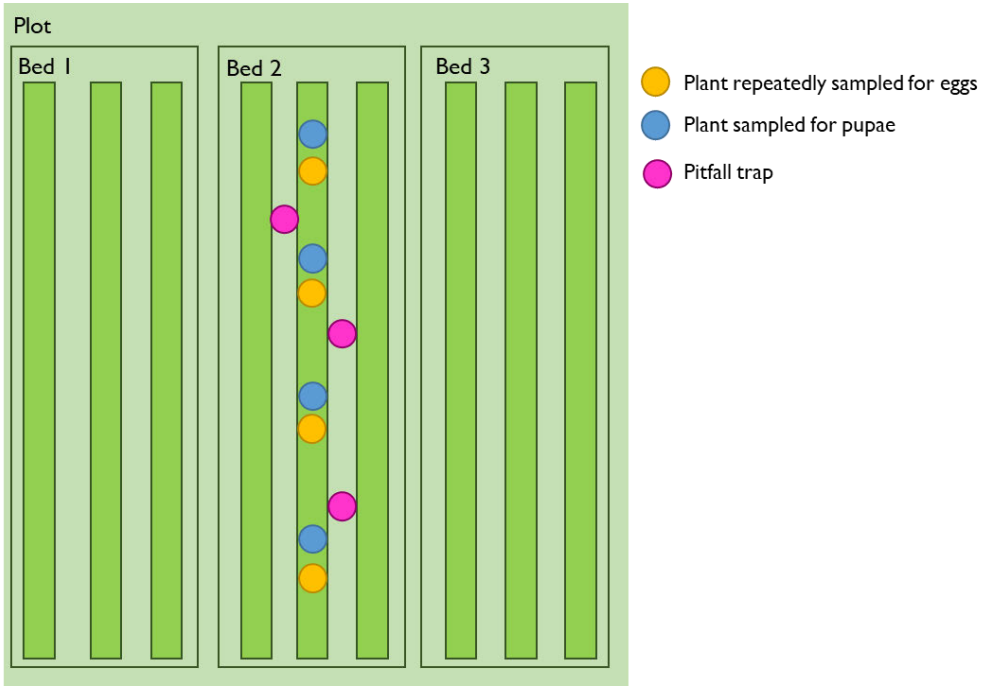


Figure 31 Sampling plan at plot level for Nafferton

Table 26 Levels of replication for field monitoring in Nafferton

	2015	2016
Egg samples	4 plants per plot x 4 blocks x 4 combinations of treatments (OPOS, OPCS, CPOS, CPCS) x 3 sampling events per generation n=192 over one generation	4 plants per plot x 4 blocks x 2 combinations of treatments (OPOS, CPCS) x 3 sampling events per generation n=96 over one generation
Pitfall traps	No pitfall traps	3 traps per plot x 4 blocks x 2 combinations of treatments (OPOS, CPCS) x 3 sampling events per generation n=72 over one generation
Pupae samples	4 plants per plot x 4 blocks x 4 combinations of treatments (OPOS, OPCS, CPOS, CPCS) x 1 sampling event per generation n=64 over one generation	4 plants per plot x 4 blocks x 2 combinations of treatments (OPOS, CPCS) x 1 sampling event per generation n=32 over one generation

Table 27 Sampling dates for Nafferton

Year	2015		2016	
Generation	1st	2nd	1st	2nd
Egg sampling	11/06, 18/06, 25/06	22/07, 28/07, 04/08	09/06, 16/06, 22/06, 04/07	10/08, 17/08, 24/08
Pitfall trap sampling			12/06, 18/06, 24/06	12/08, 19/08, 26/08
Pupae sampling	08/07	24/09	10/07	10/09

Pest and antagonist sampling and extraction

Methods described in Chapter 2 were used to carry out pest and antagonists monitoring. Egg sampling was carried out at least three times per generation, in June (1st generation) and August (2nd generation), once a week over three weeks. Unlike in Kinsealy, both years' planting dates were very similar (28th May 2015, 24th May 2016), so 1st generation egg sampling was carried out approximately 3 weeks after planting. Pupae sampling was carried out again in a similar manner. Pitfall traps, of a similar design to those used in Kinsealy, were left open for 24hrs before storing the content in 70% ethanol. Three traps were used per plot, with sampling carried out three times per generation. The same categories were used to classify any potential antagonist extracted from samples. Again, due to lack of time, no damage or individual pupal weights were recorded for Nafferton. Assistance in the field was kindly provided by Rachel Chapman and Gavin Hall, from Nafferton Farm.

3.2.6 Statistical analysis

The same methods were used to analyse our results as in Kinsealy. Count data was analysed using a GLMM allowing for Poisson distribution, with log as a link function. Random models of the GLMM reflect the trial design (fully factorial: block/crop protection*soil fertility/plot, otherwise block/plot) and sampling strategy (year/generation). Once again, no p value adjustments were put in place following statistician advice, apart from our last correlation. For the overall comparison results of this chapter, site was also included in the fixed model and effect sizes were reported where

relevant. Again, all analyses were carried out with Genstat 16 (version 16.1.0.10916, 64 bit edition, VSN International, 2013).

3.3 Results

The first part of the Nafferton field monitoring result section covers the overall analysis of the different counts, comparing fully organic to fully conventional plots and including both years and both generations. The second part considers results in more details by also including the factorial elements for 2015 sampling and considering year specific effects. The third part considers the pest and antagonist community elements from both sites.

3.3.1 Overall field monitoring analysis

Fully conventional and fully organic samples were analysed together over the two years and the two generations periods to provide an overall picture of practice impacts. Results are presented by variable measured.

Fly eggs numbers

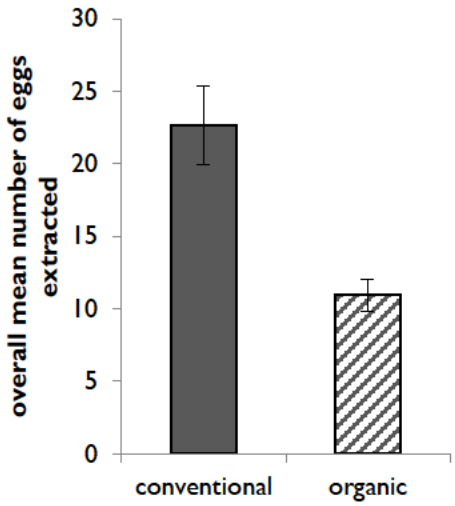


Figure 32 Overall means of egg counts for Nafferton (±SEM) – fully conventional and fully organic plots only

Similar to Kinsealy, organic soil management negatively impacted egg numbers overall (organic= 10.94±1.12, conventional=22.64±2.69, Soil F=48.64, df=274.9, p<0.001, Figure 32) with a mean reduction of 52% compared to conventional management, stronger than in Kinsealy (Kinsealy conventional= 15.74 ±0.87, organic=11.18±0.59, reduction of 29%) The count of predators extracted from those egg samples were also included in the model but had no significant impact at 5% (Total predators F=2.93, df=277.1, p=0.08)

Presence of predators in egg samples

With very similar means to Kinsealy, no significant difference was found in the presence of predators in egg samples (conventional=0.37±0.05, organic=0.38±0.06, soil F=0, df=283.1, p=0.972). Egg numbers from the same sample were also included in the model but had no significant effect (Egg numbers F=1.96, df=108.8, p=0.164). Once again, predators were not present in all 40 mL egg samples. In terms of groups, the majority of what was found was again medium Staphilinid beetles (*Aleochara* size) as shown in Table 28.

Table 28 Total counts of predators by family, size and site extracted by floatation from the 40mL egg samples.

		Kinsealy	Newcastle	
Carabid	small	29	20	Overall Carabid
	medium	21	14	
	Total	50	34	84
Staphylinid	small	72	17	Overall Staphylinid
	medium	213	77	
	total	285	94	379

Pupal numbers

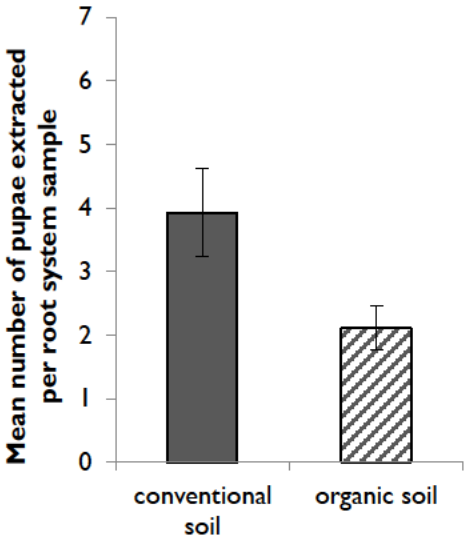


Figure 33 Overall means of pupae for Nafferton (±SEM) – fully conventional and fully organic plots only

Overall analysis of Nafferton pupae counts revealed a significant impact of soil treatment (conventional= 3.92±0.69, organic= 2.11±0.35, Soil F=9.6, df=144.8, p=0.002,Figure 33), with a reduction of 46% in the number of pupae found in organic soil. Pupae means were almost half of those extracted in Kinsealy overall where no difference was found (Kinsealy conventional= 7.94±0.90, organic= 7.41±0.67)). Predator counts from the same samples also had a significant impact on pupal numbers (Total predators F=5.53, df=145.9, p=0.024).

Root system predators

Unlike in Kinsealy, predator counts from the root system were not significantly affected by soil treatment (conventional=0.87±0.15, organic=0.80±0.17, Soil F= 0.13, df=143.1, p=0.715) and means were drastically lower than in Kinsealy's root systems as shown in Figure 34. Similar to Kinsealy however, root system predators were significantly impacted by pupal numbers within the same sample (Pupal numbers, F=4.38, df=114.6, p=0.038).

Nafferton rotation configuration and blocking structure are very different than those in Kinsealy, as the strips containing cabbages are perpendicular to semi-natural habitat, as such including location of sample to investigate potential landscape spill-over had to be carried out differently. Blocking structure was used instead of individual sample location. Block 3 was the closest to semi managed hedgerows, whereas block 1 was the closest to the field gate. For root predators, blocking structure did not have a significant effect (Block F=1.98, df=139.4, p=0.12) even with a higher mean for block 3 as shown on Figure 35.

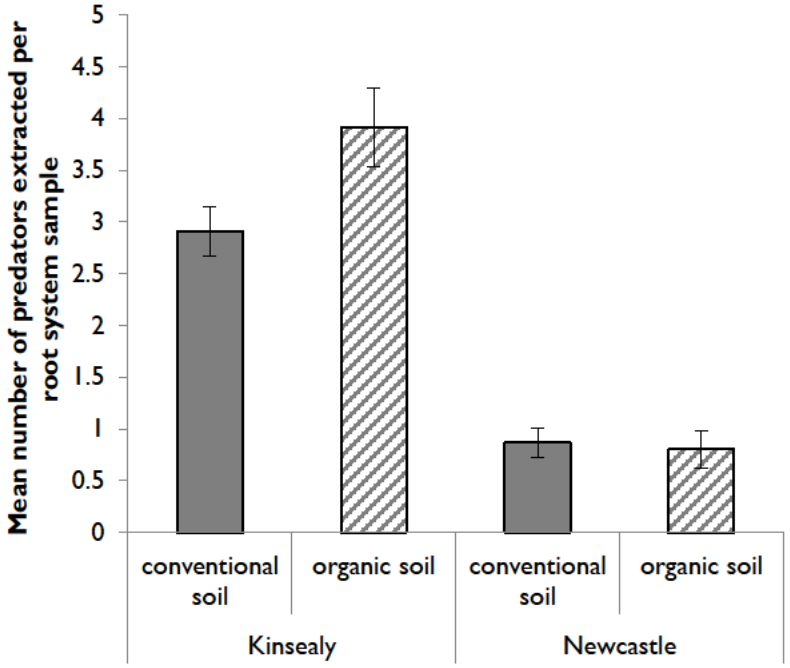


Figure 34 Overall mean root system predators (±SEM) extracted from both Kinsealy and Nafferton

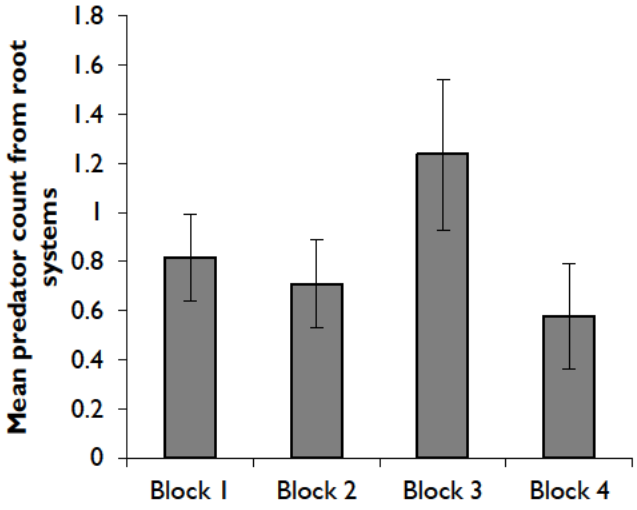


Figure 35 Mean predator count extracted from root system (±SEM), per block, in Nafferton

As in Kinsealy, the majority of predators extracted from root systems belonged to the medium Staphylinid group, as shown in Table 29, even though numbers were strongly reduced compared to Kinsealy.

Table 29 Total counts of predators extracted from root systems by family, size and site

	Kinsealy		Newcastle		
Carabid small	90	total Carabid	4	total Carabid	Overall Carabid
Carabid medium	55	145	25	29	174
Staphylinid small	160	total Staphylinid	4	total Staphylinid	Overall Staphylinid
Staphylinid medium	809	969	35	39	1008

Epigeal predator activity density

Soil management had a significant impact on activity density of epigeal predators as sampled with pitfall traps, contrasting with Kinsealy, with a higher activity density in organic plots (conventional=6.31±0.66, organic=11.19±1.37, Soil F=27.47, df=173.9, p<0.001). Average egg per plot however did not have an impact on this activity (Average eggs per plot F=0.47, df=132.8, p=0.492). Landscape impact as translated by the blocking structure had also a significant impact but unlike the trend for root systems predators, activity density was reduced in block 1, near the field gate, and not increased in block 3, close to the semi managed hedgerow as shown in Figure 36.

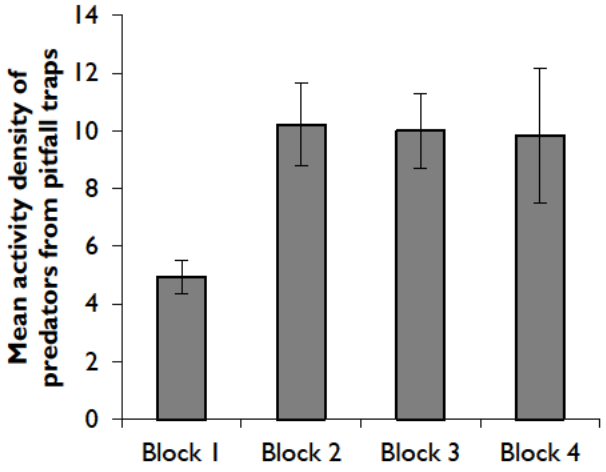


Figure 36 Mean activity density of predators extracted from pitfall traps in Nafferton (±SEM)

Pest and antagonists' correlation

A simple correlation was run including all co-occurring pest and antagonists and is summarised in Table 30. In terms of variables, as pupal numbers and root system predators

are the overall results of one generation, total number of eggs per plot per generation, as well as total pitfall trap predators per plot per generation were used.

Table 30 Correlation between pest and antagonist counts for both 2016 generations in Nafferton - Pearson correlation factor (p value), non-significant correlations in grey

Pupae	1	-				
Root system predators	2	0.238 (0.05)	-			
Total eggs per plot	3	-0.042	0.3171(0.01)	-		
Total pitfall predators	4	-0.117	-0.12	-0.031	-	
Total egg predators	5	0.111	0.126	0.295 (0.018)	0.021	-
		1	2	3	4	5

Similar to Kinsealy, egg predators were positively correlated with eggs, while root system predators were positively correlated with pupae. Here eggs and root system predators were also positively correlated. Surprisingly perhaps, pest eggs and pupae were not significantly correlated in Nafferton. In terms of community links, pitfall trap activity density was not correlated with any other variables in the system.

3.3.2 Focussing on particular sampling periods

Crop protection impacts: pesticide impact

One of the main differences in Nafferton field management compared to Kinsealy is the use of the pesticide chlorpyrifos aimed at *D. radicum*. To investigate the impact of this pesticide, sampling events and dates of application were considered together, and sampling dates were recoded as pre and post application for statistical analysis. Regarding egg numbers (Figure 37 and Figure 38), the only significant effect was found in 2016, where chlorpyrifos application seemed to have a positive effect on egg numbers during the 1st generation only (pre-post chlorpyrifos, F=8.44, df=187, p=0.004, effect size + 0.20±0.05). No clear impact was found on the very few predators extracted from egg samples. Epigeal predators' activity density, measured in 2016 only (Figure 39), was also significantly reduced after the application of chlorpyrifos (pre-post chlorpyrifos, F=129.15, df=187, p<0.001. effect size -0.69±0.06).

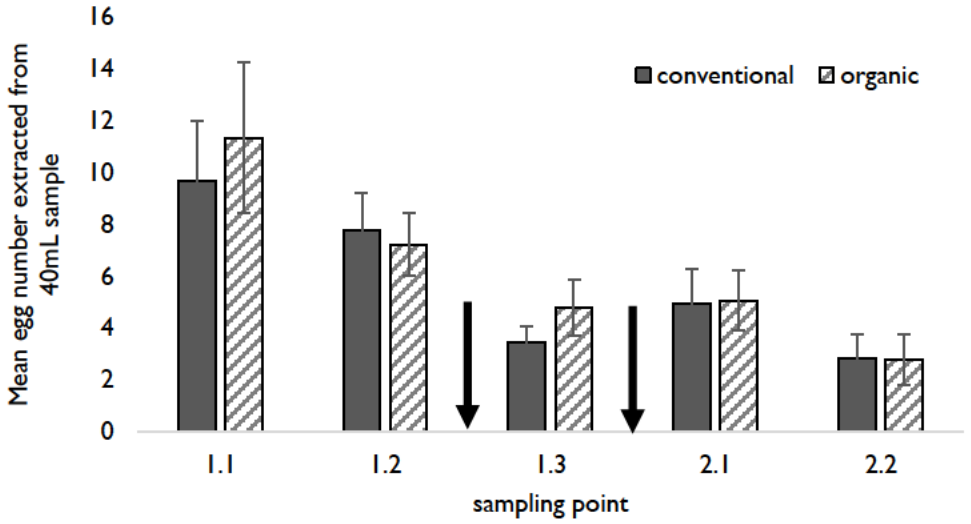


Figure 37 Mean egg numbers (±SEM) for both generations 2015 (3 sampling points during 1st generation, 2 during 2nd generation), with black arrow representing chlorpyrifos application

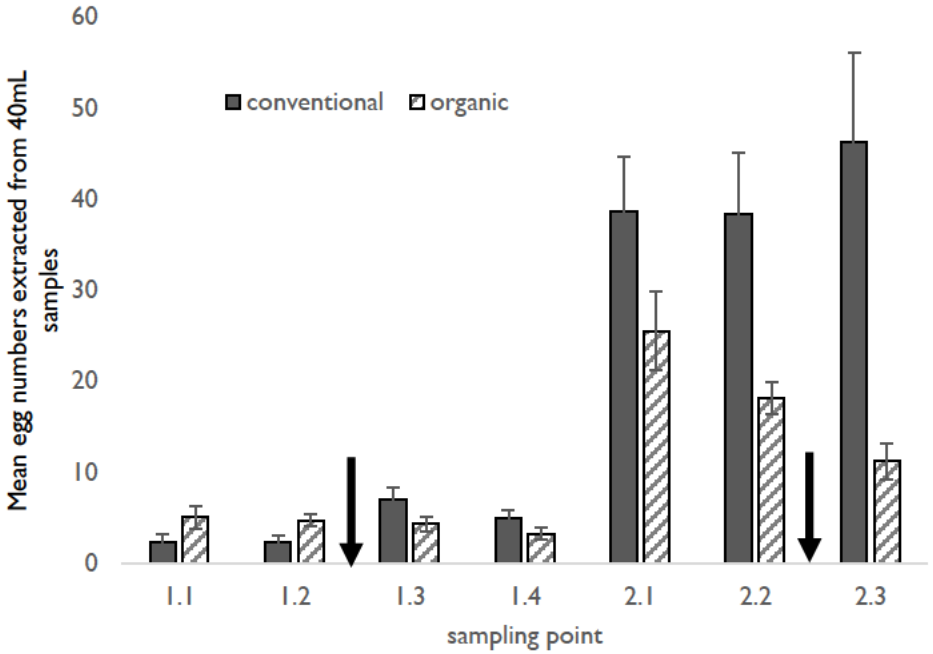


Figure 38 Mean egg numbers (±SEM) for both generations 2016, with black arrow representing chlorpyrifos application

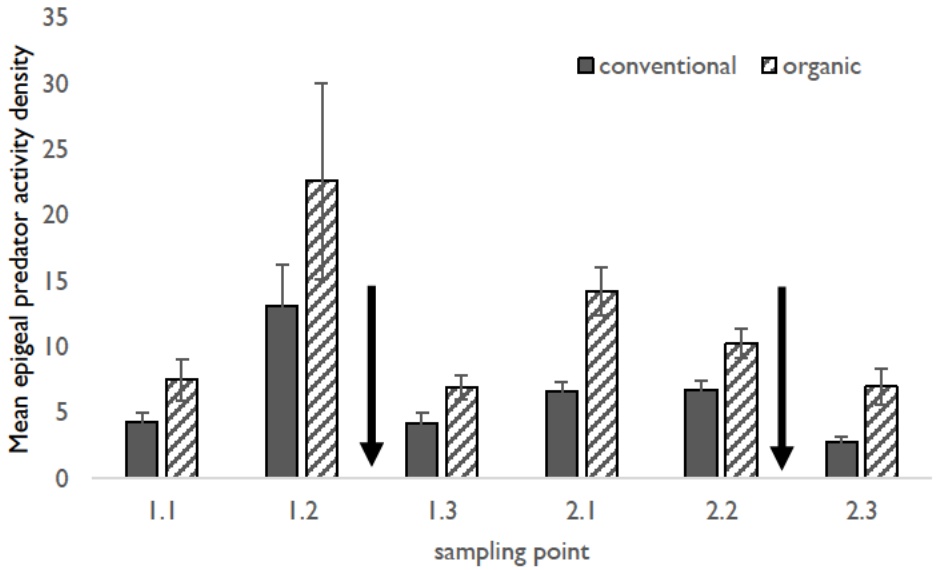


Figure 39 Mean epigeal predators activity density (±SEM) over both generations in 2016, with black arrow representing chlorpyrifos application

2nd generation build up and variations across years

Figure 40 shows the mean numbers of pupae extracted from root systems across different years and generations. Unlike in Kinsealy, there was no consistent 2nd generation build-up of fly population over the two full years sampled. The large variation in pupal numbers was not reflected in the variation of mean predators extracted from root systems, which was overall very low compared to Kinsealy (Figure 41). 1st generation of 2015 only yielded 11 pupae from 128 root systems, even when egg numbers at the start of the generation were comparable to 1st generation 2016.

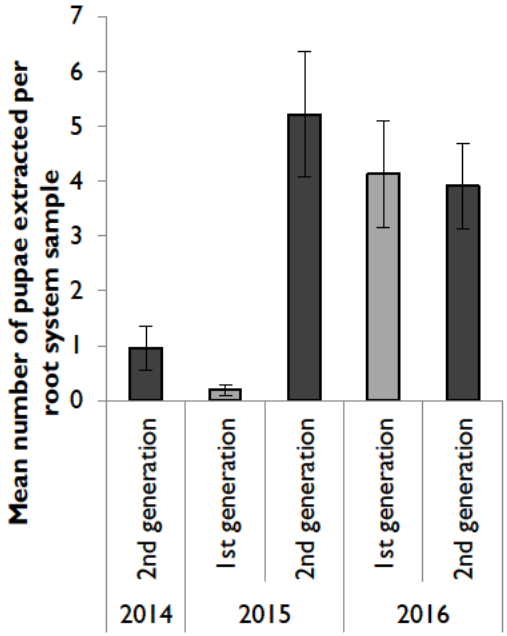


Figure 40 Mean number of pupae (±SEM) extracted from root systems for Nafferton

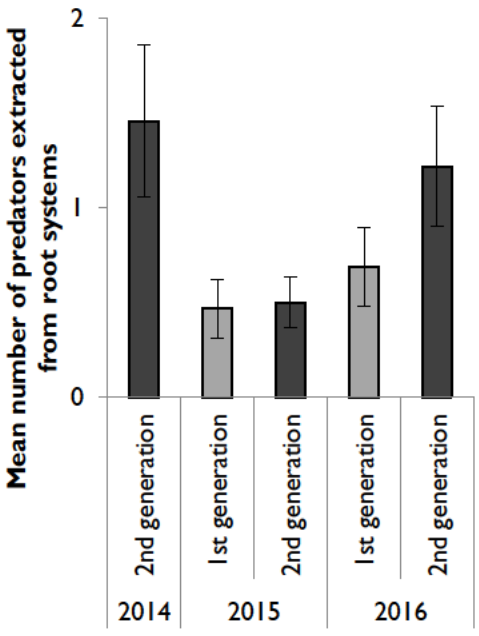


Figure 41 Mean number of predators (±SEM) extracted from root systems for Nafferton

3.3.3 Comparing sites and communities

In this section, our Kinsealy and Nafferton sites were analysed in parallel or together, in order to gain knowledge of possible underlying dynamics present in both systems, also gaining from greater statistical power. Enabling us to consider the bigger picture by including both rotations, it is worth noting that this increased power is gained at the cost of reduced precision. For clarity, we included in Table 31 a summary of the main results for both individual sites.

Table 31 Summary of field monitoring results by variable for both sites

		Kinsealy (2014-2015)	Newcastle (2015-2016)
Egg samples	Eggs	Organic< conventional (-29% in organic plots) Egg predators NS	Organic<conventional (-52% in organic plots) Egg predators NS
	Eggs predators	Similar numbers in both sites – one predator present every three samples – NS Medium Staphylinid dominant Egg numbers NS	
Root system samples	Pupae	Soil has no overall effect Root system predators have a significant effect 2nd generation build up Both years have similar generation patterns	Organic soil<conventional soil (with pesticide) Root system predators have a significant effect No consistent 2nd generation build up 2015 and 2016 have different generation patterns
	Pupae predators	Conventional soil<organic soil Pupae number has a positive significant effect	Soil NS Strong reduction compared to Kinsealy Pupae number has a positive significant effect
Pitfall trap catches		Organic protection<conventional protection Conventional fertility<organic fertility But overall soil NS Average eggs per plot has a significant effect Fewer medium beetles in conventional plots Extra predators more numerous later in the season and increase of large beetles only in conventional plots	Conventional soil<organic soil Average eggs per plot has no significant effect Overall more small and medium beetles caught than in Kinsealy Large beetles and extra predators more numerous later during growing season
Pest and predators' correlations		1.Resource-consumer link: eggs and egg predators positively correlated, pupae and pupae predators positively correlated, 2.Pest suppression link: opposite result as pitfall trap activity density is positively correlated with pupal numbers. 3.Pest pupae positively correlated with pest eggs at the start of generation 4.Even if average egg per plot has a significant effect on pitfall trap catches, total egg per plot is not correlated with activity density from pitfall traps. 5.Community links: Sampled at the same time, pitfall trap activity density and egg predators are positively correlated	1.Resource-consumer link: eggs and egg predators positively correlated, pupae and pupae predators positively correlated, 2.Pest suppression link: here pitfall trap activity density is not correlated with pupal numbers 3.Pest pupae not correlated with pest eggs at the start of generation 4.Community links: no correlation between pitfall trap activity density and egg predators but positive correlation between egg numbers and pupae predators

Kinsealy exclusion attempt summary

- More predators found in root samples when eggs present
- Fewer pupae retrieved in organic plots
- By reducing the number of predators, cages and pitfall traps might have contributed to the increase in pupae number
- Pupa/egg reduced by higher inoculation level but not by soil nor variety

Predators community comparison

Pitfall traps are a simple way of evaluating the activity density of epigeal invertebrates. But they may not reflect activity belowground when considering conservation biocontrol of a root pest. It is important here to compare abundance and diversity of trap invertebrates with those found co-occurring in our root pest samples. Too few *Delia* antagonists were found in egg samples for meaningful comparison between the soil surface and belowground habitats. However, predators extracted from root systems, potentially attracted by the presence of the pest, as earlier positive correlations have shown, can be compared to pitfall trap catches. Figure 42 and Figure 43 compare results of both sampling techniques on a Log_{10} (x + 1) scale for both sites. Both diagrams are similar in general shape, showing that both sites have similar sample compositions, even if Nafferton root systems yielded fewer predators than Kinsealy's. Nafferton pitfall traps also contained more small Staphylinid and more medium Carabid than Kinsealy's. The first obvious difference between sampling can be found in the larger beetles counts. Numerous in pitfall traps, no large Staphylinid or Carabid were extracted from root systems in either sites. Extra predators were also less abundant in root systems than in pitfall traps. Both sites' root systems communities were dominated by medium Staphylinid, which was not the case for the pitfall trap community. Table 32 reports means of both main predator families found in the different samples on both sites to compare activity of those families between soil treatments as well as between samples, as Eyre (2009) found Carabid to be more active in organically fertilised plots and Staphylinid more active in conventionally fertilised plots.

Table 32 Total Carabid and Staphylinid found in different samples on both sites (mean±SEM)

		Nafferton		Kinsealy	
		organic	conventional	organic	conventional
Egg samples	Carabid	0.12±0.03	0.08±0.02	0.06±0.01	0.03±0.01
	Staphylinid	0.23±0.05	0.27±0.04	0.26±0.03	0.27±0.03
Root systems	Carabid	0.29±0.08	0.25±0.08	0.28±0.05	0.36±0.07
	Staphylinid	0.25±0.09	0.37±0.09	3.02±0.32	2.15±0.20
Pitfall trap	Carabid	6.49±0.82	3.61±0.52	2.06±0.26	2.36±0.53
	Staphylinid	2.66±0.59	1.09±0.14	1.21±0.27	0.73±0.14

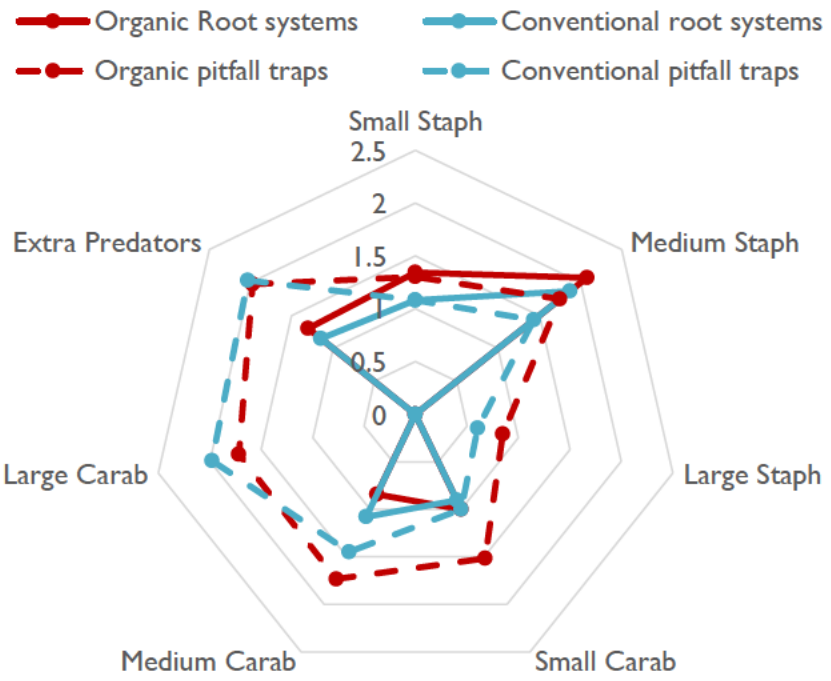


Figure 42 Total root systems and pitfall traps predators from Kinsealy 2014, compared on log+1 scale

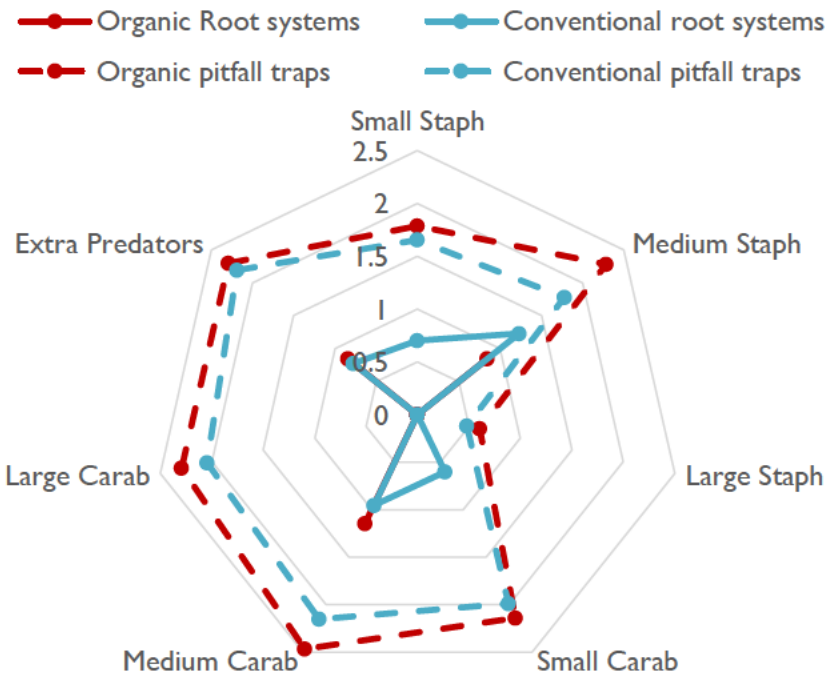


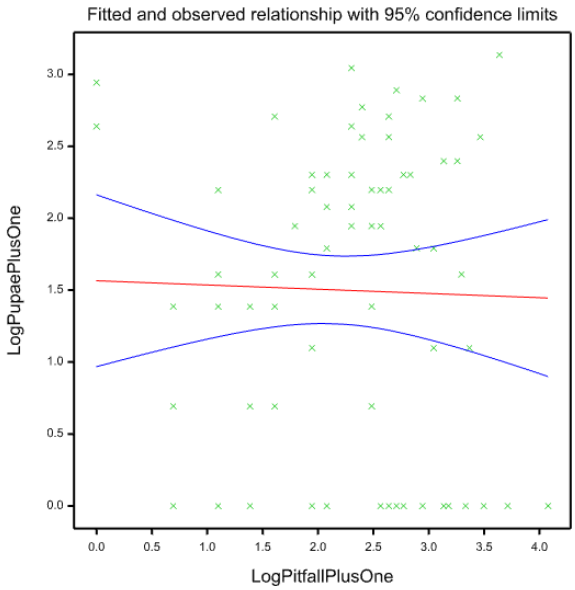
Figure 43 Total root systems and pitfall traps predators from Nafferton 2016, compared on log+1 scale

To investigate the possible negative impact of pitfall trapping on our systems, more specifically on the root system predators, we can compare figures from years including pitfall traps with figures from years without. This will however only provide a partial answer as obviously weather and other environmental parameters not included in our study will also impact figures differently depending on the year. Table 33 shows years with pitfall traps did not have consistently fewer root system predators extracted, compared to the year without traps. Strength of correlation between pupae and root predators was reduced in Kinsealy during the year including traps, however in Nafferton, 2015 correlation was not even significant compared to 2016 with pitfall traps (1st generation 2015 in Nafferton was atypical with very few pupae extracted overall). No clear predator removal impact can be identified using this simple comparison.

Table 33 Root system predators means (±SEM) for both sites and years, with correlation between pupal numbers and root system predators

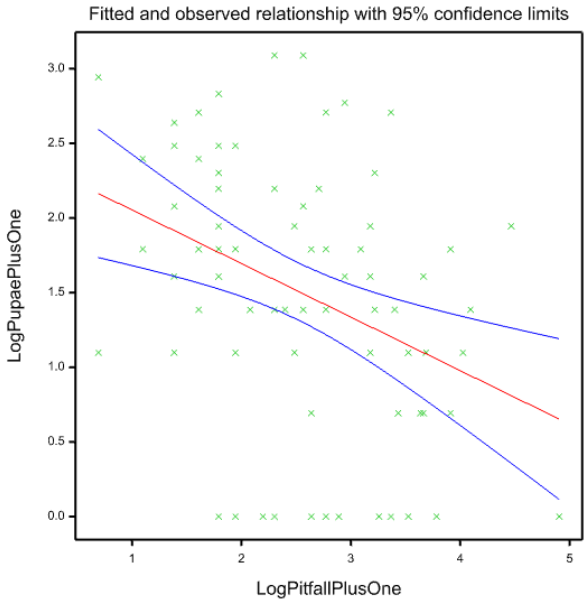
		Root systems predators		Correlation between pupae and root system predators for both soils
		conventional	organic	
Kinsealy	2014 (with pitfall traps	3.59±0.50	2.70±0.41	r^2=0.23, p=0.02
	2015	2.89±0.29	5.16±0.61	r^2=0.37, p<0.001
Newcastle	2015	0.47±0.13	0.50±0.16	r^2=0.18, p=0.16
	2016 (with pitfall traps)	1.13±0.29	0.78±0.25	r^2=0.24, p=0.05

Pitfall trap activity was assessed before pupal numbers in the root system, therefore it can be concluded that in plots where activity density was higher, pupal numbers were lower later on. We can investigate this potential pest suppression relationship further by using a simple linear regression on pupae counts and pitfall trap activity density, in both soils in parallel. Figure 44 shows the result of the regression run on conventional soils, using the log+1 scale for both variables, which is not significant. Figure 45 shows the same analysis carried out for organic soils, showing this time a significant relationship with a negative slope between pitfall trap activity density and pupae count in organic soils. Restricting the dataset in different ways can potentially help us to pin point pupae suppression by predator groups caught in the pitfall traps. Restricting the dataset by removing the large beetles from the pitfall trap data dampened the negative slope (-0.23±0.08 compared to -0.36±0.11) and decreased the variance accounted for from 12.1% to 4.4% (p=0.005). Restricting the dataset one step further by only including small and medium beetles had the same effect, decreasing the strength of the slope (-0.27±0.07) and still did not improve the variance accounted for (8.9%, p<0.001). Restricting the pitfall trap dataset to the most abundant predator groups found in root systems did not improve the pest suppression link.



Residual variance exceeds variance of response variate, vr=0.05, p=0.819

Figure 44 Regression Log(pupae+1)=f(Log(pitfall Trap+1) for conventional plots for both sites, with 95% confidence interval



Percentage variance accounted for 12.1, vr=11.51, p=0.001

Parameter	estimate	s.e.	t(65)	t pr.
Constant	2.413	0.283	8.52	<.001
Log Pitfall+1	-0.359	0.106	-3.39	0.001

Figure 45 Regression Log(pupae+1)=f(Log(pitfall Trap+1) for organic plots for both sites, with 95% confidence interval

Overall soil and predator impact on pest

Pest and antagonist activities from both sites were analysed together in GLMMs and results are reported in Table 34. The GLMMs without pitfall traps in the model include two years of data per site, whereas only one year of data could be included in the GLMMs considering the effect of pitfall traps. Even if those GLMMs cannot be compared like for like, they can still inform the different pest and antagonists links and soil management impacts over the sampled years.

Egg numbers were significantly reduced in organic soils over both sites, with fewer eggs overall in Nafferton. Overall, more egg sample predators were extracted from samples with more eggs (positive effect size). However, when pitfall trap activity density is included in the model this link disappears, whilst pitfall trap activity density has a small but significant negative impact on the egg numbers. This contrasts with the lack of correlation between egg numbers and pitfall trap activity density in **Error! Reference source not found.**. Soil management still has a significant impact on egg numbers however organic management this time seems to increase egg numbers compared to conventional treatment (positive effect size). When pitfall traps activity density is included, the interaction between soil and egg predators becomes significant, with a non-negligible F ratio, translating the fact that those predators do have a dissimilar effect on egg numbers in organic and conventional soils.

Pupal numbers were also significantly reduced in organic soils overall, with fewer pupae in Nafferton. Once again, more predators were extracted from root systems with more pupae which must have a dissimilar impact on pupal numbers, as shown by a significant soil*root predator interaction. For the single year with pitfall traps, when pitfall trap activity density is included in the fixed model, the analysis reveals a small but significant negative effect of those trap predators on the pupal numbers, similar to that for the eggs, whereas the soil treatment becomes non-significant and resource consumer link is preserved.

Table 34 GLMMs for pest and antagonists including both sites

Variable	GLMM	GLMM table				Effect sizes
Eggs numbers	Fixed model: Soil*egg predators+site Random model: Year.generation/block	Fixed term	d.f.	F	P value	Soil: conventional: baseline, organic: -0.459±0.016 Egg predators: 0.079±0.015 Site: Kinsealy: baseline, Nafferton: -1.42±0.053
		soil	1	763.79	<0.001	
		egg predators	1	52.06	<0.001	
		soil.egg predators	1	0.08	0.779	
		site	1	699.92	<0.001	
Eggs numbers with pitfall traps (2014 Kinsealy and 2016 Nafferton)	Fixed model: soil*egg predators+pitfall trap predators+site Random model: generation/block	Fixed term	d.f.	F	P value	Soil: conventional: baseline, Organic:0.221±0.03 Pitfall trap predators: -0.004±0.001 Site: Kinsealy: baseline, Nafferton: 0.40±0.041
		soil	1	38.27	<0.001	
		egg predators	1	0.12	0.733	
		soil.egg predators	1	45.38	<0.001	
		Pitfall trap predators	1	17	<0.001	
		site	1	94.3	<0.001	
Pupal numbers	Fixed model: Soil*root system predators + site Random model: Year.generation/block	Fixed term	d.f.	F	P value	Soil: conventional: baseline, organic: -0.333±0.047 Root system predators: 0.071±0.011 Site: Kinsealy: baseline, Nafferton: -1.17±0.078
		soil	1	3.81	0.051	
		Root predators	1	214	<0.001	
		soil.root predators	1	9.85	0.002	
		site	1	225	<0.001	
Pupal numbers with pitfall traps (2014 Kinsealy and 2016 Nafferton)	Fixed model: soil*root system predators+pitfall trap predators+site Random model: generation/block	Fixed term	d.f.	F	P value	Root system predators: 0.057±0.014 Pitfall trap predators: -0.006±0.003 Site: Kinsealy: baseline, Nafferton: -0.277±0.102
		soil	1	1.31	0.253	
		root system predators	1	56.02	<0.001	
		soil.root predators	1	0.77	0.38	
		Pitfall trap predators	1	21.12	<0.001	
		site	1	7.41	0.006	

3.4 Discussion

3.4.1 Overall Nafferton results

Organic management in Nafferton successfully suppressed the pest overall and outcompeted pesticide

In Nafferton, organic management had a consistent negative impact on *D. radicum* overall, reducing both eggs and pupal numbers. Egg numbers were reduced by 52% and pupal numbers were reduced by 46%, outcompeting the conventional management which included chlorpyrifos, the insecticide targeting *Delia*. This result contrasts with our Kinsealy site, where only egg numbers were reduced in open field organic soils whilst pupal numbers were only reduced in the cage setting. Pupal numbers were reduced in Nafferton organic soil even when drastically fewer root system predators were extracted than in Kinsealy, and those predators present were not significantly impacted by management. The epigeal predator activity density however was significantly higher in organic plots, again contrasting with Kinsealy and was reduced here near the field gate. The enhanced epigeal community activity density in Nafferton organic plots could potentially have participated to the reduction in pupal numbers, unlike in Kinsealy where no positive impact of organic management was detected for epigeal predators' activity density and pupal numbers.

Organic management led to lower egg numbers

Organic management used in Nafferton led to an overall reduction in egg numbers, compared to the conventional management, once again contrasting with the lack of difference found in similar study from Meyling (2013) who also compared organic and conventional management impacts on *D. radicum* at plot level. Reduction was stronger in Nafferton with -52%, compared to -29% in Kinsealy. Oviposition and egg predation cannot once again be disentangled as discussed in the previous chapter, however no result in Nafferton seems to point towards a consistent attractive or repulsive oviposition effect of management here. Unlike in Kinsealy, no chicken manure was used in Nafferton, and research has shown that cattle slurry contains no detectable DMS and DMDS compared to chicken manure (Hobbs et al., 2004).

In terms of egg predation potential, no significant difference was found in the numbers of predators present in the egg samples, but they were significantly correlated with egg numbers from the same sample, showing that those predators' presence was not fortuitous. The large proportion of medium Staphylinid in those samples confirm once again

the importance played by this group as egg predators (Wilde, 1947; Andersen et al., 1983; Fournet et al., 2000; Prasad and Snyder, 2004, 2006a). They also dominated the root system antagonist community in both sites. Contrasting with our Kinsealy site, average egg per plot had no impact this time on the epigeal predators' activity density, which was higher in organic plots, thus failing to show a link between pest egg presence and epigeal predators activity density.

Predation potential may have been reduced by the negative impact of chlorpyrifos on Nafferton natural enemies, which in turn led to an increase in egg numbers in conventional plots. Known to be detrimental to spiders, especially Linyphiid present in our system (Fountain et al., 2007), research has reported dissimilar impacts on other generalist predators with one study reporting no negative impacts (Funderburk et al., 1990) while another study reported a negative impact on Carabid, including *B. lampros* and *T.quadristriatus*, ubiquitous in our samples (Asteraki et al., 1992). Chlorpyrifos can also have a negative impact on the soil microbial activity (Fang et al., 2009) and Collembola (Eisenhauer et al., 2010), although orders seem affected differently (Michereff-Filho et al., 2004), which could in turn negatively impact the rest of the food web. As chlorpyrifos half-life is of approximately 3–14 days on the soil surface (Barron et al., 1995) and 7 to 9 days in the soil (Pandey et al., 2004), this might help explain the transient negative impact of this product in our system.

Reflecting on our sampling strategy, the sharp increase in egg number mid-generation in conventional plots during the 1st generation of 2016, as well as the inter-year variations from both sites, lead us to believe than three weekly egg sampling events per generation might be too limited to paint an adequate picture. Other field studies involving *Delia* have monitored egg presence for a month (Kergunteuil et al., 2014; Meyling et al., 2013), a month and half at different rates (Björkman et al., 2007), or 12 weeks (Nilsson et al., 2012). Investing more resources in egg sampling, especially whilst keeping the factorial elements of crop protection and soil fertilisation, similar to Eyre et al. (2009) would have helped disentangle the co-occurring ecological processes and product impacts in Nafferton. Keeping track of flowering times of wild umbellifers such as *Anthriscus sylvestris,* which provides resources for the adult flies (Nilsson, 2011), and other local landscape changes would also have improved our sampling, instead of mainly relying on pest forecasting. This highlights one of the issues of monitoring a site without being based locally.

Organic management led to lower pupal numbers without enhancing the presence of predators in the root systems

Pupal numbers were significantly reduced in organic plots, similar to Meyling (2013), even when conventional treatment included chlorpyrifos, showing that organic crop protection can outcompete conventional chemically-based crop protection at plot level. Concurrently, predator numbers in the root systems were not enhanced in organic soil whereas epigeal predators' activity densities were, contrasting with Kinsealy where we found the opposite result whilst pupal numbers were only reduced in organic management in cage settings. If chlorpyrifos had a potentially detrimental effect on predators as discussed earlier, whilst organic management tends to lead to a richer food web (Birkhofer et al., 2008a; Campos-Herrera et al., 2015; Macfadyen et al., 2009; Mäder et al., 2002; Poveda et al., 2006) and in Nafferton tends to lead to a more biologically active soil (Orr *et al.*, 2011; Gilliland 2016, unpublished data), we would have expected an enhanced presence of predators in organic root systems. However this potentially richer food web has been shown to have limited effect on some predator groups, such as Carabids whose activity did not increase with increased Collembola activity in compost fertilised crops (Garratt et al., 2011). It has to be noted that, in Nafferton, predator numbers in root systems were drastically lower than in Kinsealy, as well as pupal numbers, despite similar epigeal predator activity density. To our knowledge, no other *Delia* field study included the sampling of root system predators co-occurring with pupae or any subterranean natural enemy sampling, so we are not able to compare our results with published studies and can only highlight the different functioning of our experimental sites. Inter-year variability was strong for pupal count, and the very low count of 1st generation 2015 could not be explained (Figure 40) simply by a reduced number of eggs that generation. This again highlights the importance of investing in several years of field data collection for any pest natural regulation studies.

As mentioned earlier, Kinsealy rotation only included broccoli plots during the sampled growing season with the rest of the plots staying fallow, whereas the Nafferton rotation included a full array of crops. Those other crops were not sampled during our study but previous research in Nafferton had shown the crop and boundary preferences of Carabid beetles, with only *B. lampros* preferring vegetables plots (Eyre et al., 2013, 2012), as reported in Table 23 and Table 24. We can hypothesise that the combination of available aboveground resources in other neighbouring plots with the reduced number of pupae in

the root system led to the reduction of predators' number in the root system. However, similarly to Kinsealy, those present were still attracted by the presence of pupae, as the positive correlation in those samples shows.

Pitfall traps

As expected thanks to previous beneficial invertebrates studies in Nafferton (Eyre et al., 2013, 2009; M.D. Eyre et al., 2011), epigeal predator activity density was impacted by management, with an increased predator activity density in organic soils. Unlike in Kinsealy however, average pest egg count per plot did not have a positive impact on this activity, even with numerous egg predator *B. lampros* present. Contrasting with root predators, landscape did this time have an impact and the most disturbed part of the field (block 1) saw a reduction in activity whereas proximity to hedgerows did not have a positive impact (block 3), contrary to the expected spill over effect (Bartual et al., 2019; Gabriel et al., 2010; Holland et al., 2020; Mchugh et al., 2020; Tscharntke et al., 2007). As 2016 sampling did not include the full factorial systems, and activity densities between organic and conventional plots are not independent, the reduction in activity density of epigeal predators in conventional plots cannot be clearly linked to the use of pesticide overall, even though analysis over time showed the transient negative impact of chlorpyrifos (Figure 39)

3.4.2 Site comparisons

Predators community comparison

Commonly used in *Delia* regulation studies to assess the local antagonist community (Björkman et al., 2010; Meyling et al., 2013; Nilsson et al., 2012), pitfall trap sampling needs careful analysis. With well-known limitations and bias for larger body size (Hancock et al., 2012; Spence, 1994), this sampling technique also tends to misrepresent the activity of beneficial invertebrates. Relevant for our system, one study in arable fields reveals that in general, abundance of Carabid and Lycosid were overestimated by pitfalls, while Staphylinid and Linyphiid were underestimated (Lang, 2000). In our systems, it is clear that large beetles were overrepresented and medium Staphylinid were underrepresented in those traps, compared to antagonists found co-occurring with the two pest life stages (Figure 42 and Figure 43). Furthermore, as the extra "predators" category included surface dwelling Opiliones as well as Linyphiid and Lycosid spiders, it is not surprising that this category is also underrepresented in root systems compared to pitfall traps. Apart from its adult flying stage, *D. radicum* spends the rest of its lifecycle in the soil, starting on or close

to the soil surface at the base of the host plant, then migrating deeper in the root system (Hughes and Salter, 1959; Finch, 1989) Thus, sampling the soil surface habitat for antagonists which need to find the pest underground might seem of somewhat limited relevance, even though it is common practice in published research. Even when both habitat communities overlap, previous research has shown the predation of root pest as measured by gut content analysis was three times more frequent in the soil column than on the soil surface (Lundgren et al., 2011). Our results show a great overlap in community between pitfall trap and root systems, even if the groups used here limit our understanding compared to complete species level data. However, those communities were impacted differently both by landscape and management in both sites. Those differences strongly highlight the need for complementary sampling techniques when considering belowground trophic dynamics (Harwood et al., 2005; Luck et al., 1988; Lundgren et al., 2011; Weber et al., 2009) and not only pitfall traps that can only evaluate change and not population densities (Melbourne, 1999; Mitchell, 1963). Other techniques could include Tullgren funnels (Spence, 1994), fenced pitfall traps (Holland et al., 1999), and litter bags (Prasifka et al., 2007). In order to evaluate natural enemies of *D. radicum*, Mitchell (1963) used a combination of quadrats, capture-recapture, soil sampling, as well as pitfall traps, which, even when obviously very resource intensive, provided a complete picture of the two focus species *B. lampros* and *T.quadristriatus*. In our study, the strongest argument for actually sampling root systems and not just soil surface is the negative correlation between the two samplings here. With a several week lag complicating the relationship, we cannot conclude decisively and can only investigate deeper by comparing years with and without pitfall traps (Table 33) .

Apart from sampling two different parts of the habitat, our sampling strategy also suffers from a timing and replication issue. Firstly, pitfall traps were sampled at the same time as eggs, whilst root systems were sampled three to four weeks later. Secondly, pitfall trap sampling was repeated three times per generation, open for 24 hrs at least, unlike the root system that were just sampled once at the end of each generation. The nature of those samples also differs. Antagonists had to actively fall into the traps over 24 hrs to be sampled, leading to activity bias (Holland et al., 1999; Melbourne, 1999), whereas root predators were removed from their soil habitat instantly. If we were to redesign our sampling strategy, apart from obviously sampling both years instead of one, we would include and analyse more root systems sampling over the entire generation.

Investigating elusive pest suppression links

Conservation biocontrol studies have shown the benefit of a wide range of habitat manipulations to enhance natural enemies and reduce pest incidence, but fewer have actually identified a significant pest suppression link (Furlong et al., 2010). In our systems, sampling limitations came both from the method itself and its timing as well as the replicated plot design within the same field, preventing independence of samples. As such, our attempt at investigating a potential pest suppression link across our sample set using a regression between pupae number and pitfall traps is only an attempt to go further than ubiquitous resource-consumer links, rather than a solid proof of this suppression. With those limitations in mind, the overall analyses including both sites with and without pitfall traps are perhaps more telling. When pitfall trap predators were added to the overall pupae analysis, soil management lost its significant impact on the number of pupae, unlike when considering eggs. Tentatively, this could point towards the suppression potentially being linked to predators mainly, more than to the soil-based suppression carried out by smaller taxa, entomopathogens as well as bottom up control. Chapter 4 investigates those in more details.

3.5 Conclusion

In Nafferton, organic management reduced both eggs and pupal numbers, outcompeting the pest suppression from dedicated pesticide used in conventional management. While the few predators extracted from the root systems were not impacted by management, epigeal predators were more active in organic plots, as expected from previous Nafferton studies. Resource-consumer links were found once again between co-occurring pest and antagonists across our different samples, making the possibility of a pest suppression link harder to investigate. Inter-year variability was present but no contrasting effect was found between years. Nafferton organic management suppressed *D. radicum* more strongly than Kinsealy, where only egg numbers were reduced in open field, and not pupae. Links between pest and antagonist variables differed between sites, but resource-consumer links were common in both systems. Pitfall trap sampling of antagonists did not reflect abundance and composition of antagonists found co-occurring in pest samples accurately, highlighting the need for the use of complementary sampling techniques. Whilst perhaps not being the most appropriate way of assessing *D. radicum* predators, pitfall traps did reveal however the only pest suppression links in our systems, specifically through small

and medium Carabid beetles as well as medium Staphylinid beetles. Soil management impacts were drastically changed when this key variable was added to overall pest analysis. This last analysis would point towards the major role played by predators in pest suppression compared to microfauna-based suppression, carried out by microorganisms, along with bottom up control from a more resilient organic crop in both trials. This microfauna-based suppression and its bottom up counterpart are the subject of Chapter 4 investigations.

Chapter 4 Soil and microfauna-mediated natural regulation of root pests

4.1 Introduction

Farming practices impact the soil biodiversity across taxa, including pest antagonist communities and microbial biodiversity (Altieri, 1999; Altieri and Nicholls, 2003; Altieri and Nicholls, 2004; Gurr, Wratten and Altieri, 2004; Zehnder et al., 2007; Birkhofer et al., 2008; Rusch et al., 2017). Conservation biocontrol studies and reviews have so far focussed mainly on predators and parasitoids and their habitat manipulation (Landis et al. 2000; Gurr *et al.*, 2004; Straub, Finke and Snyder, 2008; Nilsson et al., 2016; Begg et al., 2017; Gontijo, 2018; Shields *et al.*, 2019), with a more limited number of studies including entomopathogens (Lewis et al., 1997; Landis et al., 2000; Klingen and Haukeland, 2007; Meyling and Eilenberg, 2007; Stuart et al., 2008; Pell et al., 2010; Campos-Herrera, El-Borai and Duncan, 2015). In the context of integrated pest management, research has also shown than soil is more than just a reservoir for pest antagonists contributing to top down regulation (Kaya et al., 2012; Klingen et al., 2006; Lacey et al., 2015; Meyling et al., 2007, 2006).Those same antagonists can also help plants fight pathogens and herbivores (Vega et al., 2009) and even promote plant growth (Barelli et al., 2016; Behie et al., 2012; Lacey et al., 2015), alongside other beneficial microbial organisms contributing to improve bottom up control (Alyokhin et al., 2019; Kupferschmied et al., 2013; Magdoff, 2007; Sturz et al., 2003).

Soil management has also been shown to impact pest herbivory, with contrasting impacts of mineral and manure based fertilisation (Alyokhin et al., 2005; Eigenbrode et al., 1988; Letourneau et al., 1996; Meyer, 2000; Phelan et al., 1996; Scriber, 1984). Concurrently, entomopathogens are also impacted by soil management and an important body of research can help us identify beneficial practices for entomopathogenic fungi (Clifton et al., 2015; Goble et al., 2010; Klingen et al., 2002; Klingen et al., 2006; Meyling et al., 2011; Ramos et al., 2017; Tkaczuk et al., 2014; Uzman et al., 2019), as well as entomopathogenic nematodes (Campos-Herrera et al., 2010, 2008; Klingen et al., 2006; Williams et al., 2013), with only limited information on entomopathogenic bacteria and viruses (Lacey et al., 2001, 2015; Lacey, 2012).

The study of impacts on *Delia* of entomopathogenic nematodes (Beck et al., 2014; Leger et al., 2009; Nielsen et al., 2004; Richardson et al., 2002; Willmott et al., 2002) and fungi (Bruck et al., 2005; Chandler et al., 2005; Shulong Chen et al., 2003; Klingen et al., 2002; Vanninen et al., 1999) has improved our understanding of its microbial control and more recent research also started to decipher belowground interactions between soil biota and *D. radicum* (Ourry et al., 2018; Razinger et al., 2014) helping improve our understanding of root ecosystems, which can contribute to improved pest suppression strategy.

After investigating the overall impact of the entire *D. radicum* antagonist community and focussing on the mesofauna present in our two experimental field sites in Chapter 2 and 3, this chapter focusses on the microfauna part of this community, its impact on the root pest and includes as well as soil management wider impact on the plant-soil-pest system in controlled conditions. In this chapter, with the exception of predatory mites, all predators and parasitoids were removed from the system, to focus on the antagonistic microorganisms as well as the wider beneficial soil microbial community of brassica root systems. Soils surrounding field brassica root systems were used in two complementary experiments, including both sites, years and generations. Soil baiting using *Galleria mellonella* larvae (Meyling, 2007) was carried out to assess the overall suppression potential of organic and conventional soils through the entomopathogen community, compared to a sterile control. The same soils were also used for the investigation of the plant-soil-pest system in an inoculation experiment in controlled growth chamber conditions.

As organic management has been shown to foster soil biodiversity, microbial activity and microorganisms beneficial to plants (Birkhofer et al., 2008a; Fließbach et al., 2007; Henneron et al., 2014; Monokrousos et al., 2006; Reilly et al., 2013) compared to more intensive conventional management, we hypothesise that organic management will lead to a higher pest suppression and will limit the negative impact of root herbivory.

The research questions are:

- Does organic management increase model pest mortality due to entomopathogens compared to conventional management?
- How does the model pest survival vary over time between systems?

- How does soil management impact plant growth in controlled conditions?
- Does organic soil improve bottom up control of inoculated *D. radicum* in controlled conditions?

4.2 Materials and Methods

4.2.1 Assessing pathogen potential impact: soil baiting experiments

Soil preparation

Previously examined for fly pupae and predator presence which were all removed, root system soils from our experimental sites were prepared for both baiting and inoculation experiments at the same time to allow for complementarity of experiments.

Field monitoring over two years and two fly generations allowed for four soil replicates over time. Soils from both sampled fly generations from Kinsealy 2014 and 2015 as well as Nafferton 2015 and 2016 were included as separate replicates in both experiments. As pupae were extracted by floatation from the soils of the 2nd generation of Kinsealy 2014, this replicate could not be included in these experiments as all soils were washed away from the root systems, reducing the number of replicates for Kinsealy to three.

Stored at 4°C, samples were taken out of cold storage and placed in controlled temperature room for a week at 20°C to allow for the recovery of microbial community (B. Griffiths, personal communication). The objective of these experiments being linked to the overall soil management impact and not the investigation of within-field variability, plant, plot, and variety sampling levels were not included. As such, individual root system soil samples were mixed at plot level then at management level, to produce two large composite samples of organic or conventional soils. A third of both samples was subsequently mixed together and autoclaved to produce the sterile soil control.

***Galleria* soil baiting protocol**

Our soil baiting method was developed by adapting the method using the greater wax moth larvae *Galleria mellonella* (Lepidoptera: Pyralidae) described in detail by Meyling (2007), originally developed by Zimmermann (1986). This method is very commonly used in entomopathology studies (Beck et al., 2014; Clifton et al., 2015; Jabbour et al., 2009; I. Klingen et al., 2002; Meyling et al., 2006; Tkaczuk et al., 2014; Uzman et al., 2019) and is

mainly used to determine pathogen occurrence and diversity in different soils. Our aim however differs slightly. Soil baiting is used here to test the overall virulence of the soil pathogen community on the model pest, not only fungi or nematodes occurrence, and its potential contribution to pest field mortality. As such, our focus will not be the individual identification of pathogens baited but rather on the overall mortality compared to the sterile soil control as well as the survival curve of the model organism over time.

A pilot soil baiting experiment was set up using Kinsealy 2013 broccoli soils (Figure 46) with 15 larvae per tub in order to test the protocol and assess the actual quantity of work and replication needed, as well as to try the complementary statistical analysis. As heat treatment of the larvae rendered them sluggish and silk webbing was minimal without it, this heat treatment recommended in Meyling (2007) was not included in our protocol.

Food grade plastic 500 mL tubs were sterilised and their lids pierced with small holes to allow for air circulation. Tubs were labelled and filled with a standard amount of soil using a 400 mL plastic scoop. Soil moisture was adjusted, enough to produce condensation on the lid as described in Meyling's method. Ten tubs were prepared per organic, conventional and sterile soil treatment. Commercially reared antibiotics-free *Galleria mellonella* larvae (UK Waxworms Ltd) were procured the day before setting up each soil baiting repetition. The larvae were inspected for overall health and vigour, and ten average size mobile larvae were introduced in each soil tub. Replication structure is included in Table 35. Once closed, tubs were slowly turned over several times to allow larvae to be covered by soil and not just lie on the surface. In order to assess larvae batch quality, at least 15 larvae were kept as a control batch in their original sawdust packaging for the duration of the experiment and regularly checked (Figure 47). As 99% of larvae from the control batch either survived or pupated, no mortality data adjustment was required. All 30 tubs, as well as the control batch, were then placed in the dark, in a controlled temperature room at 20°C. Soil tubs were assessed for larval mortality every three days, apart from the Nafferton 2015 1st generation replicate, which was checked every day, in order to test a modified statistical analysis that was abandoned.

Table 35 Soil baiting experimental set-up

Soil treatment (3 levels)	Tub replicates	Larval replicates
Organic	10 tubs	10 larvae per tub
Conventional	10 tubs	10 larvae per tub
Sterile (half organic, half conventional, autoclaved)	10 tubs	10 larvae per tub
		3x10x10=300 larvae per experiment
+15 larvae kept in original packaging for quality control		

Figure 46 Galleria mellonella larvae in soil tub, pilot study with Kinsealy 2013 soils.

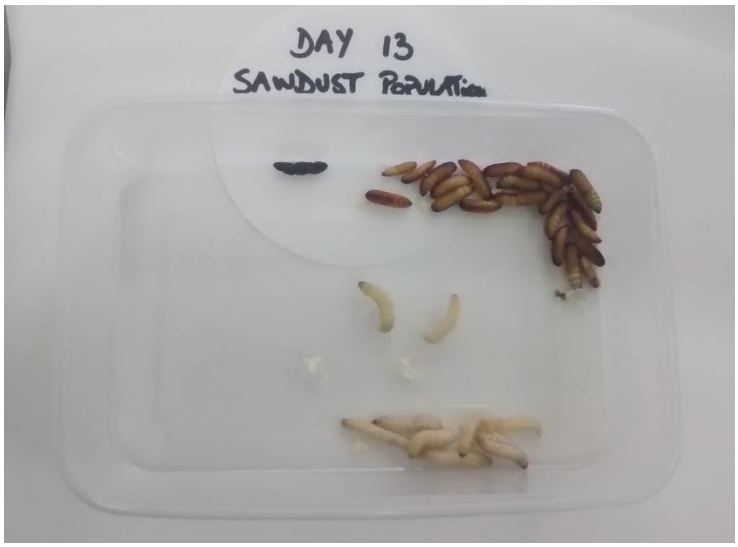


Figure 47 Batch quality control - Galleria larvae and pupae after 13 days during our pilot study, showing only one dead larva (black)

To assess larval mortality, soil tub content was tipped onto a sterilised metal tray and all 10 larvae were inspected. As larvae become less mobile just before pupation, only immobile dead larvae not responsive to touch were removed from the soil tub. Extracted with swan neck forceps, they were dipped for 10 seconds in 10% sodium hypochlorite solution to remove any surface contaminant and placed on sterile moist filter paper in a 10 cm labelled petri dish. Number of deaths and dates were recorded. Some *Galleria* larvae failed their last moult before pupating and died in their hardened cuticle (Figure 48). Unfortunately, very little information was found on this failure to moult, seldomly reported in the literature. Larvae that failed to pupate correctly were excluded from our analysis (see statistical analysis section for details).

Figure 48 Galleria failed pupae, with hardened last moult, retaining larva head and legs.

Normally formed pupae were recorded but left in the soil as they would still be susceptible to pathogens. Mortality assessment was conducted until the great majority of larvae had died or pupated and whole experiments lasted a maximum of 25 days. At the end of the experiment, all cadavers in petri dishes were assessed for nematode presence as well as fungi sporulation under 10 times magnification. Figure 49 and Figure 50 show examples of the larval state at the end of the pilot experiment, with some fungi sporulation.

Figure 49 Example of larvae at 21 days from organic soil - pilot study

Figure 50 Example of larvae at 21 days from conventional soil - pilot study

During the experiment, some larvae decayed so quickly that they completely liquefied, and could not be retrieved from the soil tubs. They were recorded as part of the mortality data but not placed in a petri dish. Bleaching the larvae also seemed to have failed to remove all external organisms present on the surface of the larvae. Some active mites were present in the petri dishes on the dead larvae, sometimes in very high numbers. Nematodes were also present in the majority of samples. As they can easily travel on wet filter paper and spread across the entire dish, no count of individually infected larvae was possible, and only presence or absence at tub replicate level was recorded. Furthermore, as no detailed identification was carried out, presence of entomopathogenic nematodes (EPN) could not

be differentiated from presence of more general bacterial feeders that commonly occur with EPN (Campos-Herrera et al., 2012). Characteristic of some EPN ambusher *Steinernema* species (Campbell et al., 2010; Grewal et al., 2009), some nictation behaviour (when a worm stands on its tail and waves its head in three dimension) was regularly noticed, as shown on Figure 51 . The only pathogen group that could be accurately recorded was the sporulating entomopathogenic fungi on individual larvae. Quick broad identification was carried out using reference material pictured during the entomopathology training received in Copenhagen university (Figure 52).

Figure 51 Nictating nematodes on soil sample around Galleria larva (x20 magnification)

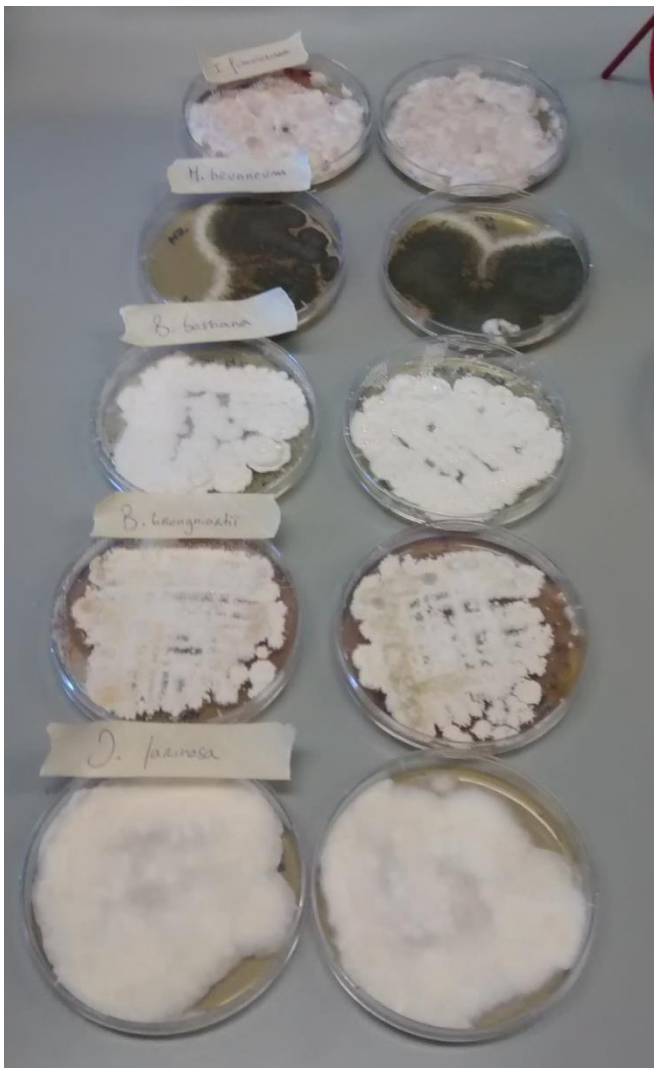


Figure 52 EPF reference plates from Copenhagen University training August 2015 (own picture). From top to bottom: **Isaria fumosorosea, Metarhizium brunneum, Beauvaria bassiana, B. brongniartii, I.farinosa**

Complementary data analysis options – overall mortality (GLMM Binomial) and survival analysis (KM survival function)

Soil baiting data was analysed in two complementary ways. The first variable analysed was a simple mortality ratio, using the proportion of dead larvae out of the ten initially introduced. This total of ten was modified if larvae died by failing to pupate. As this variable is binary (dead/alive) over a set total, a GLMM allowing for binomial distribution using a logit link was used, with modified larvae total used as binomial totals. Random model accounted for the tub replicate whilst fixed model included soil treatment. As the length of the experiments varied, this mortality ratio was calculated from values at the end of each

experiment but was also extracted at 15 days for each (apart from Kinsealy 2015 1st generation which only lasted 13 days as all larvae died) to allow for fair comparisons.

Nematode presence/absence was not included in the analysis as more than 99% of the non sterile soils petri dishes contained some nematodes. Sporulating larvae however were only seldomly present and their count was analysed using a GLMM allowing for Poisson distribution.

As larval mortality was also monitored over time, survival analysis was also possible. Commonly used in human population monitoring (see Scottish Longitudinal Study, sls.lscs.ac.uk), this stepwise function allows for comparison of survival rates of different categories of individuals over time. This approach can provide information on the virulence of the soil pathogenic community, showing how quickly larvae died and also allows for the removal of participants, labelled as "censored", needed here to remove the failed pupae. Data was formatted to fit Genstat 16 Kaplan Meyer estimate options, including time points (number of days larvae have been in the soil), number at risk (larvae introduced minus failed pupae), number of deaths and groups (soil treatments). No random model/tub replicate level could be included here, and aggregated numbers per treatment were used. As mortality was assessed every three days, time intervals were included instead of actual time points. This unfortunately prevented us from comparing the survival curves statistically, as Genstat only offers this option when using exact time points. Without being compared statistically, the survival functions for each soil baiting replicate still provide a qualitative comparison of the death rate of the *Galleria* larvae in the different soils.

4.2.2 Inoculation experiment: survival of cabbage root fly on Brassica grown in experimental field soils

Pre inoculation phase

Soil was prepared as detailed in the previous section. Seeds of "Belstar" and "Fiesta" were sourced (Thompson & Morgan products: Broccoli 'Fiesta' F1 Hybrid (Calabrese) and Broccoli 'Belstar' F1 Hybrid (Calabrese)) and germinated in half trays filled with a mix of peat, coir and vermiculite (1:1:1) at 18ºC 65% RH in a greenhouse. Ornamental industry standard 11 cm square pots were filled with a standard amount of soil to 1 cm away from the top, labelled, and one broccoli seedling was transferred to each pot

at the 1st true leaf stage. In addition to the three soil treatments (organic, conventional, sterile) and the two varieties (Belstar and Fiesta), an inoculation treatment also needed to be included, with its control counterpart (inoculated/non inoculated). For each experiment, ten replicates of each treatment combination (3x2x2x10=120 pots) were prepared and placed on the greenhouse bench following a replicated randomised block design (example of layout in Annex 10, experimental set-up in Table 36).

Table 36 Inoculation experimental set-up

Treatment	Levels and replication
Soil treatment	3 levels: organic, conventional, sterile
Variety treatment	2 levels: Belstar, Fiesta
Inoculation treatment	2 levels: Inoculated, non inoculated
	10 replicated pots per combination: 3x2x2x10=120 pots

In order to assess management impacts on soil fertility and its impact on the plants, no extra fertiliser was used. Plants were grown in greenhouse conditions (18ºC 65% RH), watered when needed, until their five to six true leaves stage. At this stage, the whole set of plants was moved into a growth chamber (18ºC 65% RH, 16hrsL:8hrsD), to allow for fly egg inoculation. Pots were replaced following the same experimental design layout than at growing stage as shown on Figure 53.

Figure 53 Experiment set up in growth chamber condition at inoculation stage

Inoculation

D. radicum eggs were taken from the dedicated SRUC insectary culture (Figure 54). Full fly rearing protocol is included in Annex 11. In summary, to provide egg laying sites, fresh organic swede cubes were placed in plastic saucers filled with sieved washed sand (<1mm) and left in the fly cages for 48hrs, as shown on Figure 54. Eggs were then carefully extracted by simple floatation and quickly placed at the base of the stem of half of the plants with a paintbrush, at the rate of 20 eggs per plant. Eggs were lightly covered in soil from the pot to avoid desiccation. Plants were left to grow for another four weeks, watered regularly and checked for other potential pests and diseases. Aphids were regularly an issue during some experiments and biocontrol agents were introduced in the growth chamber to avoid population explosion (Koppert© Aphiscout and Aphidend, every two weeks as advised). Mid experiment, some plants started to show nutrient deficiency signs as shown in Figure 55. After four weeks in the growth chamber, all plants were destructively sampled. Stems were cut at soil level and the top part of the plant was placed in labelled paper bags, and dried for 48hrs at 65ºC in a drying oven. The root system from each pot was soaked in tap water for 10 minutes to help root washing, then the root systems were carefully washed and any floating pupa extracted, counted then placed in a labelled petri dish. Due to lack of time, damage score was not assessed. Washed root systems were then dried in the same manner as the top part of the plant. Aboveground biomass and belowground biomass dry weights were assessed using a precision scale (Entris Sartorius GmbH, Goettingen).

Figure 54 Egg laying sites in local fly culture cages

Figure 55 Inoculated plants in growth chamber conditions, showing signs of nutrient deficit

Inoculation statistical analysis

The final inoculation experimental dataset consisted of aboveground biomass dry weight, belowground biomass dry weight and extracted pupae number. Each replicate over time using specific site, year and generation soil was analysed separately then regrouped and analysed per site. Dry weights were log transformed and analysed using a GLMM Normal distribution, with soil*variety+inoculation as fixed model and year/generation/block as random model. Pupae counts were analysed using the dataset

restricted to inoculated plants, using a GLMM Poisson distribution using soil+variety and year/generation as random model. Inoculation experimental variables were also analysed using a simple correlation (Pearson coefficient). All analyses were carried out using Genstat 16 (version 16.1.0.10916, 64 bit edition, VSN International, 2013).

4.3 Results

Results are presented by field sites, starting with soil baiting mortalities, sporulating larvae counts and survival curves, followed by inoculation plant and pest variables.

4.3.1 Kinsealy soils

Soil baiting: overall mortality, sporulating larvae and survival analysis

As explained above, mortality proportions were both analysed at 15 days (13 days for Kinsealy 2015 1st generation, as all larvae in organic and conventional soils were dead at 13 days) as well as at the end of the experiments. Length of experiments are reported in Table 37.

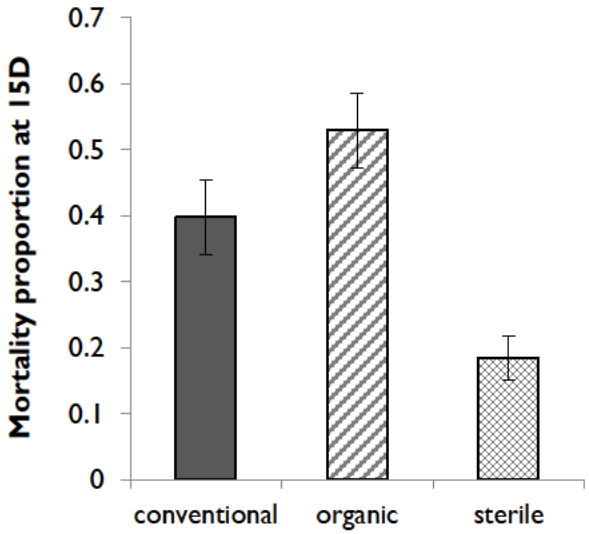


Figure 56 Mean mortality (±SEM) of model organism at 15D for all Kinsealy soils tested

Mortality of the model pest at 15 days (Figure 56) was significantly impacted by soil treatment ($F=24.43$, $df=85.3$, $p<0.001$). Mortality in sterile soil was the lowest at 18.4%±3.3%, followed by conventional at 39.8%±5.7%, with organic soil leading to the highest mortality rate at 52.9%±5.6%. Analysis on organic and conventional data only showed that organic mortality was significantly higher than conventional mortality ($F=9.14$, $df=56$, $p=0.004$)

Results ten days later (Figure 57) followed the same pattern. Mortality at the end of experiment was significantly impacted by soil treatment, with the lowest mortality caused

by sterile soil at 33.4±4.6%, followed by conventional soil at 59.0%±4.4%, with organic soil leading to 71.6%±3.7% mortality.

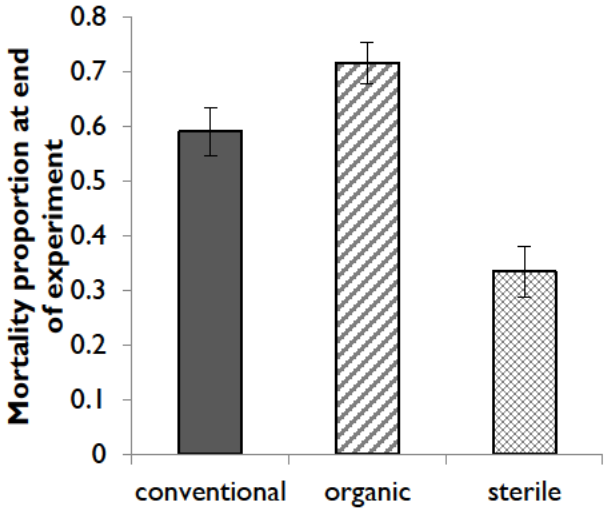


Figure 57 Mean mortality (±SEM) of model organism at the end of experiment for all Kinsealy soils tested

Table 37 Kinsealy soil replicates included in soil baiting experiments with duration

	Length of baiting experiment
Kinsealy 2014 1st generation	25 days
Kinsealy 2014 2nd generation	Pupae floated – no soil left
Kinsealy 2015 1st generation	13 days (all organic and conventional model pest dead)
Kinsealy 2015 2nd generation	21 days

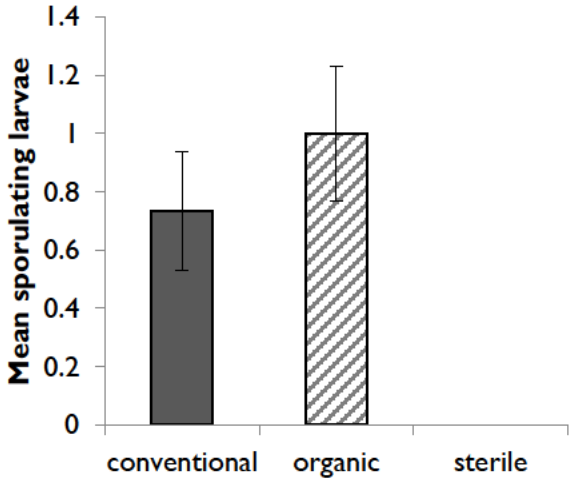


Figure 58 Mean number (±SEM) of sporulating larvae per tub at 25 days for Kinsealy soils

No sporulating larvae were found after being exposed to sterile soil (Figure 58). Number of sporulating larvae per tub replicate was very low overall, ranging from 0.73±0.20 larva out of 10 for conventional soils to 1.00±0.23 for organic soils. No difference was found between organic and conventional treatments (F=0.58, df=85, p=0.56).

Survival curves (Kaplan Meyer estimate, Figure 59) show the variability of larvae survival across treatments for the different soil replicates. In the same conditions, Kinsealy 2015 1st generation soils killed larvae quicker than the other replicates, with no difference between organic and conventional soils. Sterile soil and conventional soil had similar survival curves with 2015 2nd generation soils.

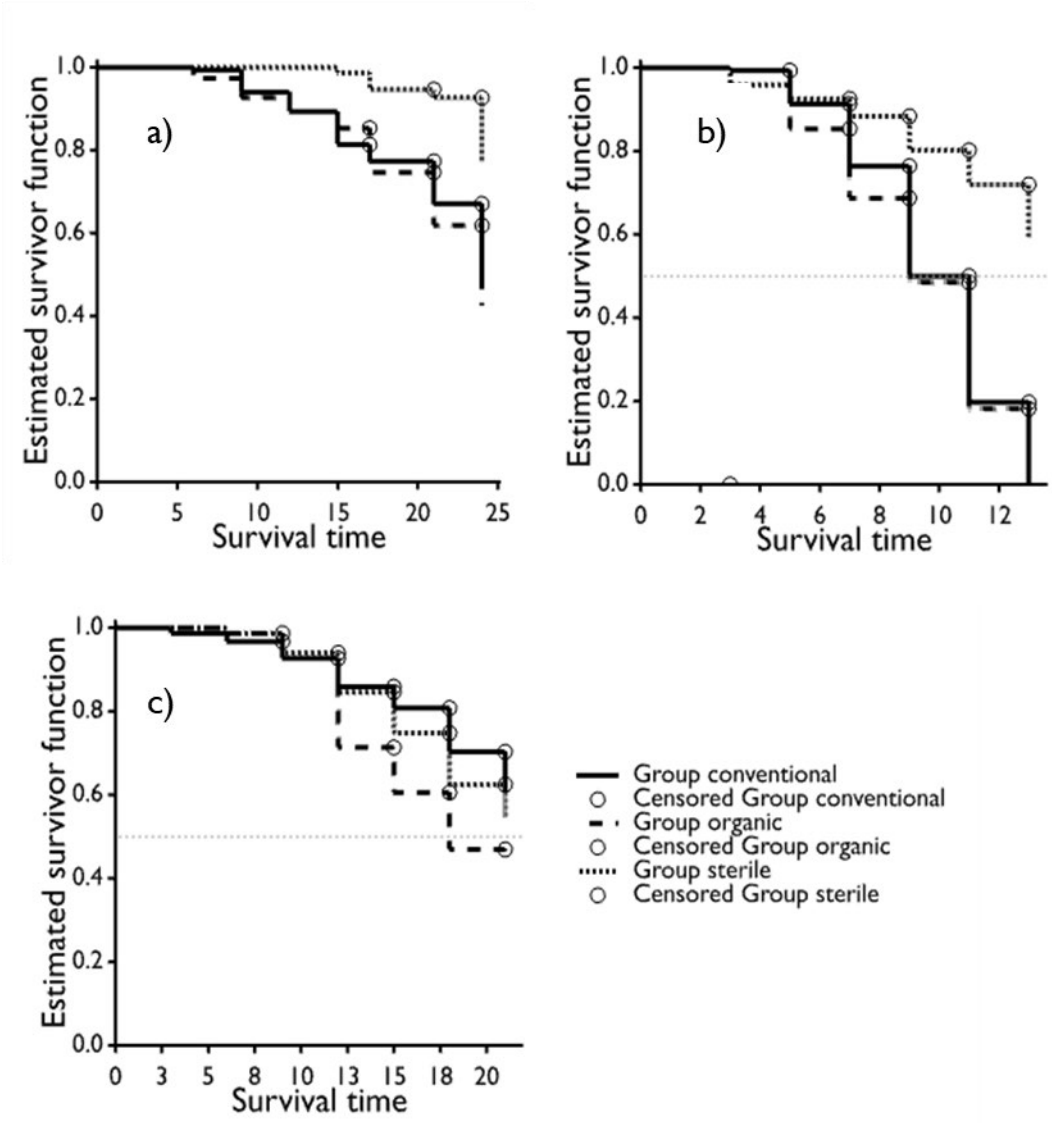
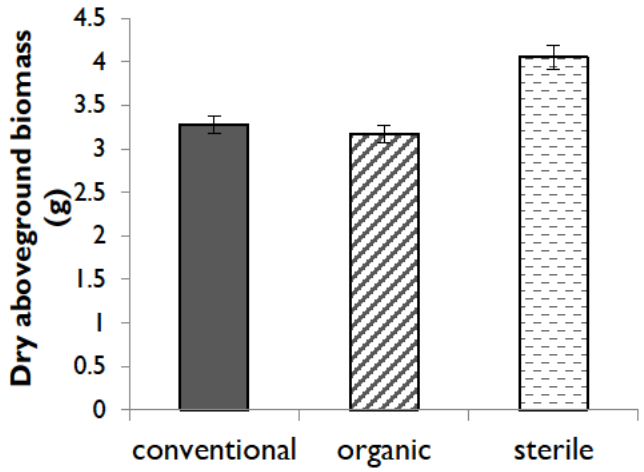


Figure 59 Survival curves (Kaplan Meier estimate) for Kinsealy soils, with censored data (failed pupae) a) Kinsealy 2014 1st generation, b) Kinsealy 2015 1st generation, c) Kinsealy 2015 2nd generation

Inoculation: aboveground biomass, belowground biomass, recovered pupae



Aboveground biomass was significantly impacted by all treatments (Figure 60). Sterile soil plants were larger than plants grown in organic and conventional soils ($F=11.7$, $df=647.4$, $p<0.001$) but no difference was found between organic and conventional soils ($F=1.61$, $df=137$, $p=0.21$).

Figure 60 Mean (±SEM) dry aboveground biomass (g) for all soil treatments at the end of inoculation experiment for Kinsealy soils

Belstar plants were significantly larger than Fiesta plants ($F=11.68$, $df=647.4$, $p<0.001$), similarly to the only significant result for stem diameter in field conditions, for 2014 1st generation plants. Inoculation also had a significant impact on aboveground biomass, with a 10% average reduction of weight for inoculated plants ($F=13.88$, $df=647.4$, $p<0.001$, inoculated=3.32±0.09g, non inoculated=3.70±0.09g) across all three soil treatments.

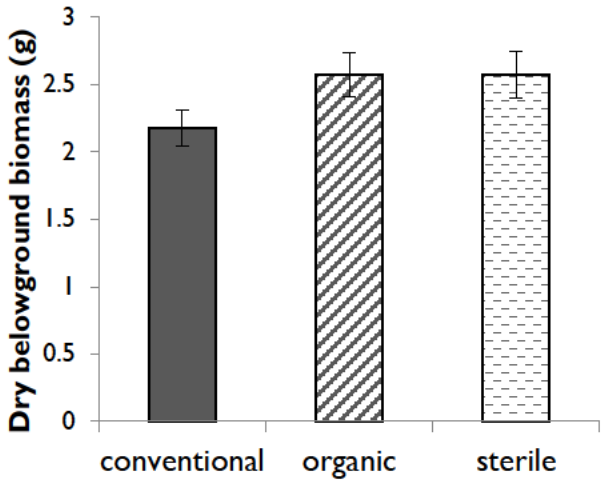


Figure 61 Mean (±SEM) dry belowground biomass (g) for all soil treatments at the end of inoculation experiment for Kinsealy soils

Surprisingly perhaps, inoculation had no significant effect on dry belowground biomass (F=0.04, df=216, p=0.84, Figure 61). Soil treatment however had a significant effect, with a 15% reduction of root system in conventional soil compared to organic or sterile soils (F=6.04, df=216, p=0.003). Variety had no significant impact here.

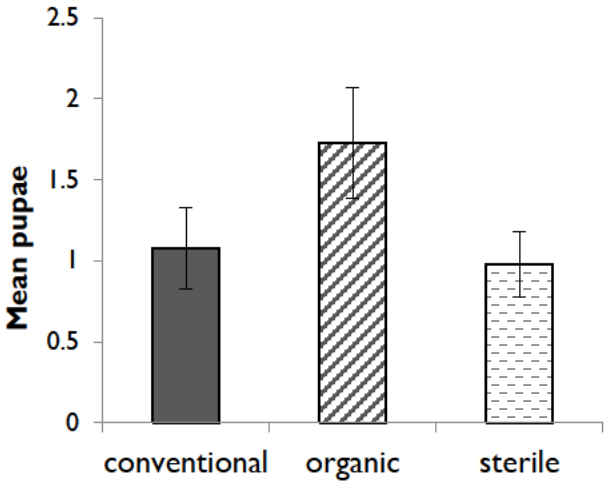


Figure 62 Mean (±SEM) pupae extracted for all soil treatments at the end of inoculation experiment for Kinsealy soils

Very few pupae were extracted from root systems overall (Figure 62). More pupae tended to be extracted from organic soils compared to sterile or conventional soil, but the difference was not significant (F=1.2, df=235, p=0.30). Variety had no significant impact either. When the dataset was restricted to non-zero values, more pupae were found in organic soils (F=4.41, df=28.3, p=0.045).

Summary table for Kinsealy soil replicates

Table 38 presents results per soil replicate over time for Kinsealy.

Table 38 Summary table of soil baiting and inoculation experiment results for all replicates of Kinsealy

	Soil baiting experiment		Inoculation experiment		
	Soil baiting mortality	Sporulating larvae	Aboveground biomass	Belowground biomass	Recovered pupae
Kinsealy 2014 1st generation	sterile < conventional and organic	no difference between organic and conventional	conventional and organic < sterile	NS	none
Kinsealy 2014 2nd generation	Pupae floated – no soil left to use				
Kinsealy 2015 1st generation	sterile < conventional and organic	Zero sporulating larvae	NS	NS	Very few
Kinsealy 2015 2nd generation	conventional < sterile and organic	no difference between organic and conventional	conventional <sterile and organic, Fiesta<Belstar,	conventional <organic <sterile, Fiesta<Belstar, inoculated<non inoculated	sterile and conventional < organic
Overall analysis all years, all generations	sterile <conventional <organic	no difference between organic and conventional	conventional and organic<sterile; Fiesta<Belstar; inoculated<non inoculated	Inoculation and variety NS; conventional <organic and sterile	NS Non-zero count: conventional and sterile<organic

Inoculation correlation

In order to consider the whole plant-soil-pest system, a simple correlation was used to highlight links between variables (Table 39). For Kinsealy, aboveground and belowground biomasses were positively correlated at r^2=0.65 and number of pupae were positively correlated with belowground biomass at r^2=0.21, even when inoculation had no significant impact on belowground biomass. The larger the root systems, the more pupae were extracted from the soil at the end of those experiments.

Table 39 Correlation between inoculation variables for Kinsealy soils (Pearson coefficient), with p value in bracket. Non-significant correlations are in grey.

Pupae	-		
Belowground biomass	0.2055 (0.001)	-	
Aboveground biomass	-0.0362 (0.581)	0.6536 (<0.001)	-
	Pupae	Belowground biomass	Aboveground biomass

4.3.2 Nafferton soils

Soil baiting: overall mortality, sporulating larvae and survival analysis

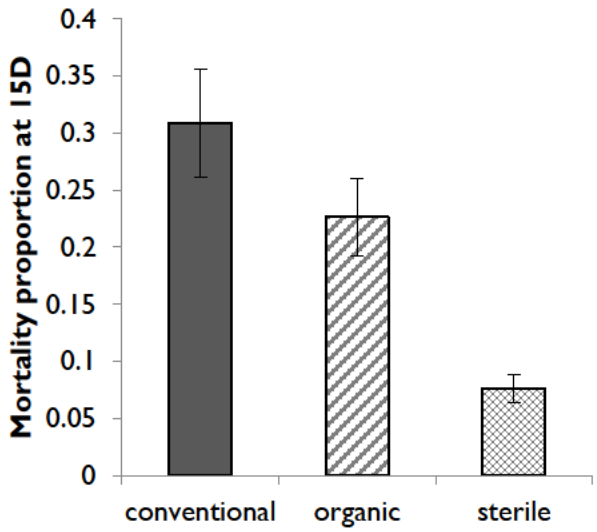


Figure 63 Mean mortality (±SEM) of model organism at 15D for all Nafferton soils

Mortality at 15D of the model pest (Figure 63) was significantly impacted by soil treatment ($F=16.17$, $df=115.1$, $p<0.001$). Mortality in sterile soils was the lowest at 7.6%±1.2%, followed by organic soil at 22.6%±3.4%, then by conventional soils at 30.8%±4.7%. Organic mortality was significantly lower than conventional mortality ($F=6.25$, $df=76$, $p=0.015$).

At the end of experiments (Table 40), sterile soil treatment still caused the lowest mortality (Figure 64) at 25.9%±3.1% compared to organic and conventional soils ($F=42.79$, $df=114.2$, $p<0.001$). However, the difference between organic and conventional soils found at 15 days disappears, with both field soils causing similar mortality (organic=65.1%± 2.9%, conventional=62.4%±4.1%, $F=0.02$, $df=74.9$, $p=0.89$).

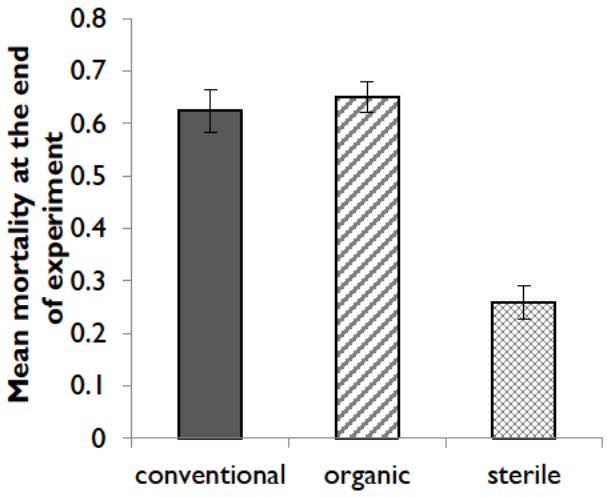


Figure 64 Mean mortality (±SEM) of model organism at the end of experiment for all Nafferton soils tested

Table 40 Nafferton soil replicates included in soil baiting experiments with duration

	Length of baiting experiment
Nafferton 2015 1st generation	15 days
Nafferton 2015 2nd generation	21 days
Nafferton 2016 1st generation	21 days
Nafferton 2016 2nd generation	21 days

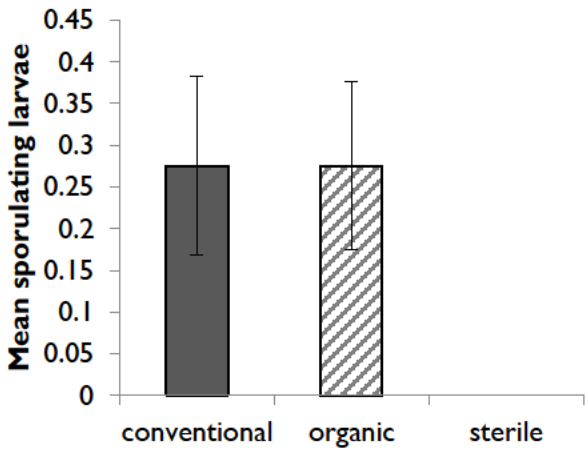


Figure 65 Mean number (±SEM) of sporulating larvae per tub at 25 days for Nafferton soil

Once again, no sporulating larvae were found in sterile soils. Counts were also very low, out of 10 larvae, only 0.27±0.11 were found in conventional soil and 0.27±0.10 found in organic soil (Figure 65). There was no significant difference between organic and conventional soils (F=0, df=2, p=0.99).

Survival curves (Kaplan Meyer estimate, Figure 66) show the variability of larvae survival across treatments for the different Nafferton soil replicates. 2015 1st generation killed larvae quicker than the other replicates. 2016 2nd generation shows the least difference in survival rate in different soils.

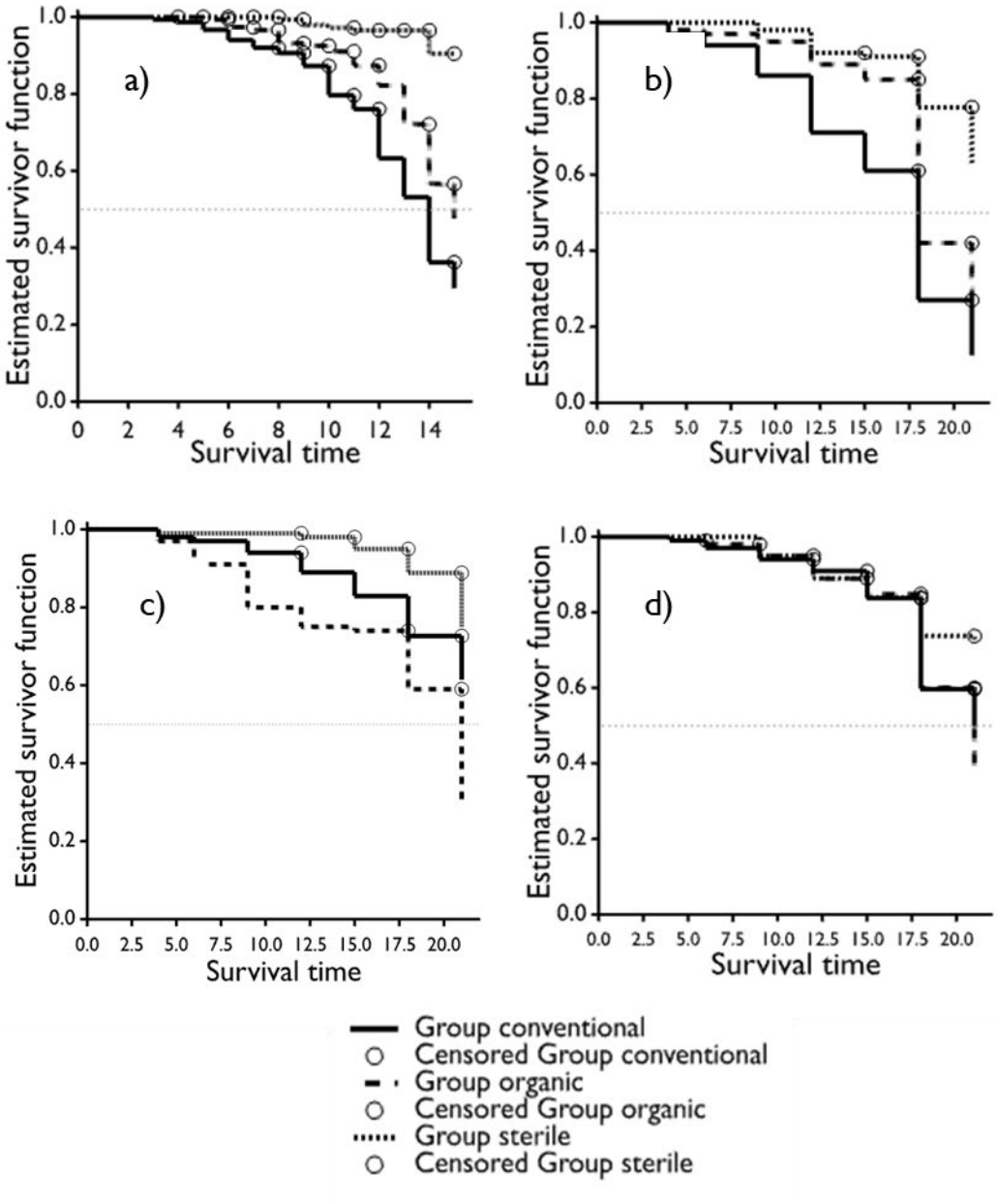


Figure 66 Survival curves (Kaplan Meier estimate) for Nafferton soils, with censored data (failed pupae) a) Nafferton 2015 1st generation, b) Nafferton 2015 2nd generation, c) Nafferton 2016 1st generation, d) Nafferton 2016 2nd generation

Inoculation– aboveground biomass, belowground biomass, recovered pupae

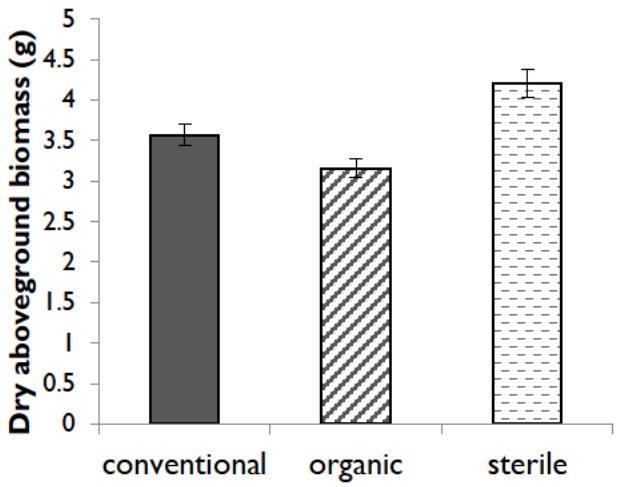


Figure 67 Mean (±SEM) dry aboveground biomass (g) for all soil treatments at the end of inoculation experiment for Nafferton soils

Aboveground biomass was significantly impacted by all three treatments (Figure 67). Soil treatment had a significant impact with sterile plants being the largest ($F=9.99$, $df=407.4$, $p<0.001$). Organic plants were 12% smaller than conventional plants ($F=4.97$, $df=264$, $p=0.027$). Belstar plants were once again larger than Fiesta ($F=9.64$, $df=407.4$, $p=0.002$) and inoculation reduced the aboveground biomass by 6% ($F=.29$, $df=407.5$, $p=0.007$)

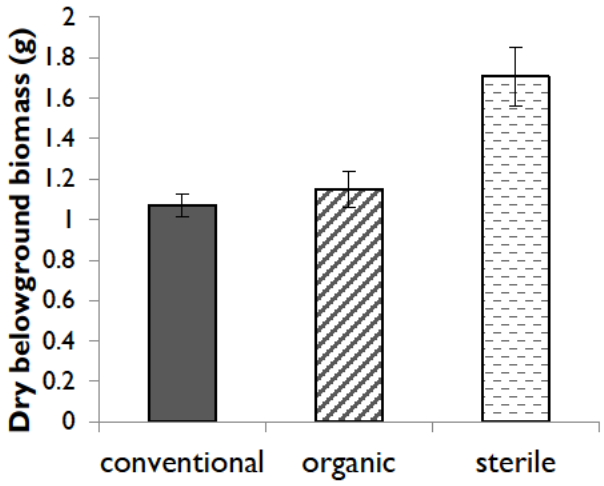


Figure 68 Mean (±SEM) dry belowground biomass (g) for all soil treatments at the end of inoculation experiment for Nafferton soils

Similarly to its aboveground counterpart, belowground biomass was also impacted by all three treatments (Figure 68). Sterile plants were larger ($F=12.36$, $df=648.8$, $p<0.001$) but no difference was found between organic and conventional root systems. Belstar had a larger root system ($F=14.27$, $df=264$, $p<0.001$) and inoculation negatively impacted the plant with a 18% reduction in weight of root system ($F=5.98$, $df=649$, $p=0.015$).

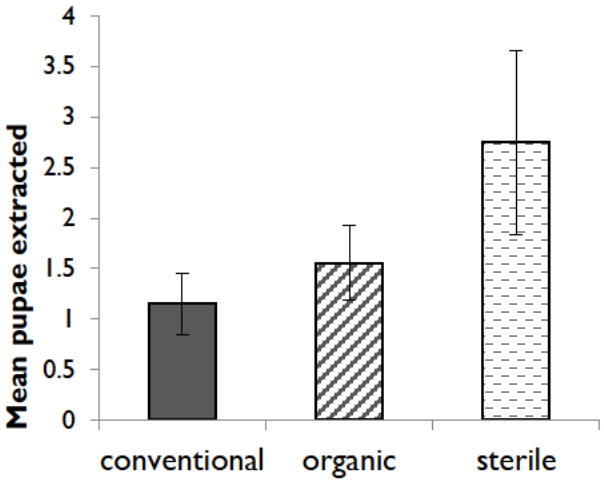


Figure 69 Mean (±SEM) pupae extracted for all soil treatments at the end of inoculation experiment for Nafferton soils

Once again very few pupae were extracted overall (Figure 69). However, this time soil and variety both had a significant impact. More pupae were retrieved from sterile soils compared to both field soils ($F=4.7$, $df=198.8$, $p=0.01$) and more pupae were recovered on Fiesta plants ($F=5.08$, $df=198.8$, $p=0.025$)

Summary table for Nafferton soil replicates

Table 41 presents results per soil replicate over time for Nafferton.

Table 41 Summary table of soil baiting and inoculation experiment results for all replicates of Newcastle

	Soil baiting experiment		Inoculation experiment		
	Soil baiting mortality	Sporulating larvae	Aboveground biomass	Belowground biomass	Recovered pupae
Newcastle 2015 1st generation	sterile<organic <conventional	none	organic <sterile and conventional; Fiesta<Belstar	organic <conventional <sterile	very few conventional <sterile, organic not different from either
Newcastle 2015 2nd generation	sterile <conventional and organic	no difference between organic and conventional	conventional and organic<sterile	conventional and organic<sterile	organic and sterile <conventional
Newcastle 2016 1st generation	sterile <conventional < organic	Only 2	inoculated< non inoculated, Fiesta<Belstar	inoculated< non inoculated,	NS
Newcastle 2016 2nd generation	sterile <conventional and organic	Only 3	organic and conventional< sterile, inoculated <non inoculated	conventional<sterile<org, inoculated< Non inoculated,	conventional and organic<sterile
Overall analysis	sterile<organic and conventional	NS	Inoculated <non inoculated; Fiesta<Belstar; organic <conventional <sterile	Inoculated <non inoculated; Fiesta<Belstar; conventional and organic <sterile	Belstar<Fiesta; conventional and organic<sterile Non zero counts: same results.

Inoculation correlation

In Nafferton, aboveground and belowground biomasses were positively correlated at r^2=0.37 (Table 42). However, pupae were not significantly correlated with belowground biomass but rather with aboveground biomass this time, at r^2=0.18. Perhaps surprisingly, an increase in number of pupae did not negatively impact the size of the top biomass, but rather, the more pupae extracted, the larger the plant.

Table 42 Correlation between inoculation variables for Nafferton soils (Pearson coefficient), with p value in bracket. Non significant correlation are in grey.

Pupae	-		
Belowground biomass	-0.0134	-	
Aboveground biomass	0.1822 (0.01)	0.3697 (<0.001)	-
	Pupae	Belowground biomass	Aboveground biomass

4.3.3 Overall analysis (both sites, both generations)

Summary table for Kinsealy and Nafferton soils

For clarity, a summary table (Table 43) across both sites is included here.

Table 43 summary table of main results for Kinsealy and Nafferton

	Kinsealy	**Nafferton**
Mortality at 15D	Sterile<conventional<organic	Sterile<organic<conventional
Mortality at the end of experiment	Sterile<conventional<organic	Sterile<conventional and organic
Sporulating larvae	None in sterile soil – very few in organic and conventional soils, with no difference (less than 1 sporulated larva per tub)	None in sterile soil – very few in organic and conventional soils, with no difference (0.27 sporulated per tub)
Aboveground biomass	Organic and conventional<sterile Fiesta<Belstar Non inoculated<inoculated	Organic<conventional<sterile Fiesta<Belstar Non inoculated<inoculated
Belowground biomass	Conventional<sterile and organic Variety NS Inoculation NS	Conventional and organic<sterile Fiesta<Belstar Inoculated<non inoculated
Pupae	Soil NS Non-zero dataset: conventional and sterile<organic	Organic and conventional<sterile Belstar<Fiesta
Correlation	Belowground biomass positively correlated with pupae r^2=0.21; Aboveground and belowground biomass positively correlated r^2=0.65	Aboveground biomass positively correlated with pupae r^2=0.18 ; Aboveground and belowground biomass positively correlated r^2=0.37

Pathogen occurrence on larvae exposed to field soil:

Dead larvae could be classified into three broad categories. The great majority of dead larvae turned a strong shade of black, similar to the bacterial infections observed in pathology studies using *Galleria* as a model organism (Ramarao et al., 2012) and some of them became liquefied very quickly. The second category of dead larvae stayed soft and the same off-white colour, even when covered in nematodes. The last category changed colour slightly, sometimes light brick colour and became hard to the touch. The majority of the last category sporulated later on and no nematodes were observed on those larvae.

Observed diversity of sporulating fungi was low, with the great majority of infected larvae displaying *Metarhizium* characteristic spore colour and texture. Possibly some *Beauvaria* and *Isaria* fungi were present but in low numbers (less than 10 over thousands of larvae used). Out of 1400 larvae exposed to non sterile soils, only 74 (around 5%) were visually identified as sporulating.

Soil sampling timing impact on plant growth and pest

Soil sampled from both fly generations were used in the inoculation experiment. As the season progresses, nutrients will be used by the crop and timing of soil sampling could potentially have an impact on inoculated broccoli growth. As Kinsealy has only one 2nd generation replicate, data from both sites was pooled together. Log transformed aboveground and belowground biomasses were analysed across sites using a GLMM Normal distribution using soil+inoculation*generation as fixed factor and site/sampling year/generation/block as random model. Aboveground biomass tended to be larger in 2nd generation soils (1st generation abg biomass=2.63±0.11, 2nd generation abg biomass =3.87±0.16) but the difference was not significant (F=0.98, df=233.1, p=0.32) and no interaction was found between factors. In contrast, belowground biomass was significantly impacted by sampling timing (F=6.84, df=232.3, p=0.01, 1st generation blg biomass =1.38±0.08, 2nd generation blg biomass =3.45±0.09), with a 60% increase in biomass for the plants grown in 2nd generation soils. No significant interaction was found between factors for the belowground biomass either.

Using a restricted dataset with only inoculated plants, pupal numbers were reanalysed across sites using a GLMM Poisson distribution with soil+generation as fixed model and site/samplingyear/generation/block as random model. Generation had no significant impact on pupal numbers (F=4.51, df=2, p=0.17)

Pupae to egg ratio

Similar to our caged experiment in Chapter 2, pupa to egg ratios were calculated to assess pest success in growth chamber conditions (Table 44).

Table 44 Pupae to egg ratios (±SEM) from inoculation experiments for both sites

	Kinsealy	Nafferton
organic	0.05±0.01	0.17±0.04
conventional	0.08±0.02	0.17±0.03
sterile	0.05±0.01	0.38±0.11
Significant difference? (GLM)	NS (non-zero dataset: sterile and conventional<organic	Organic and conventional<sterile

4.4 Discussion

4.4.1 Organic management failed to consistently increase pest suppression compared to conventional management

Both organic and conventional field soils led to a higher model pest mortality compared to their sterile counterpart but their impact on plant growth and *D. radicum* survival were not consistent across experimental sites. Compared to conventional soil, organic soil from Kinsealy consistently suppressed the model pest more strongly, led to larger brassica root systems without reducing the aboveground biomass, whilst unexpectedly allowing *D. radicum* to potentially survive better on inoculated plants. In contrast, Nafferton conventional soil suppressed the model pest more strongly than organic soil but only momentarily, led to a larger brassica aboveground biomass without producing a larger root system and had similar impact to organic soil on *D. radicum* survival on inoculated plants. The expected positive impact of organic management on plant-soil-pest interactions was not consistent within this study and dissimilarities between sites point toward contrasting soil functioning. Even though entomopathogenic nematodes impact could not be accurately assessed, they were ubiquitous in our samples, contrasting with entomopathogenic fungi, whose presence was only recorded in very low numbers, without any difference between field soils.

4.4.2 Comparing impacts of organic and conventional management on entomopathogens.

Contrary to expectation, organic soils did not consistently suppress either the model pest or *D. radicum* better than conventional soils. While Nafferton organic soil had a smaller or similar impact on *Galleria* than conventional soil, Kinsealy organic soil had a consistently larger impact on *Galleria* mortality. However, this positive impact could not be linked to an increased presence of baited pathogens. Organic management has been shown to improve soil biodiversity and microbial activity (Birkhofer et al., 2008a; Henneron et al., 2014; Mäder et al., 2002; van Diepeningen et al., 2006) but previous research has only shown limited differences between organic and conventional management impact on entomopathogens. Concerning fungi, Klingen *et al.* (2002) and Ramos *et al.* (2017) found entomopathogenic fungi more frequently in organic systems. Uzman *et al.* (2019) and Tkaczuk *et al.* (2014) only found minor differences in the impact of organic and conventional managements, whilst Clifton *et al.* (2015), Meyling and Eilenberg (2011), and Goble *et al.* (2010) found no difference. A study focussing on the effect of transition from conventional to organic on EPF over three years did not detect any trend in fungi occurence either (Jabbour et al., 2009). Overall management might not be the adequate level at which to discriminate between soils, as more specific factors seem to have a stronger impact on EPF than overall management, including tillage (Bing and Lewis, 1993; Hummel *et al.*, 2002b; Clifton *et al.*, 2015), soil physical factors (Quesada-Moraga et al., 2007), or pesticides (Mietkiewski et al., 1997). Here, chlorpyrifos which has been shown to have a negative impact on EPF (Mietkiewski et al., 1997) did not significantly reduce the occurrence of fungi baited in Nafferton conventional soils. Compared to fungi, fewer studies have investigated differences between systems on entomopathogenic nematodes, with some revealing a positive impact of organic management (Campos-Herrera et al., 2010, 2008; Williams et al., 2013) or no significant effect (Jaffuel 2016). Other more specific factors impacting EPN have been investigated (see review in Klingen and Haukeland 2006), including soil texture (Koppenhöfer et al., 2006), crop type (Jaffuel et al., 2016) and soil habitat conditions (Hoy et al., 2008). Here we at least removed the variation in texture between field soils and control, by using sterilised field soils instead of standard growing media or sterile garden centre soil (Uzman et al., 2019), where texture would have differed greatly. Specific soil factors evaluated at the time of soil baiting, such as standard

commercial soil analysis available to farmers, would have potentially offered more perspective on the observed lack of difference between soil managements.

4.4.3 Low presence or low detection of fungal pathogens?

Entomopathogenic fungi occurrence, recorded as sporulating larvae, was very low overall, with only 74 larvae out of 1400 exposed to non-sterile field soils. The low diversity of fungi was not surprising as other surveys including numerous different sites have only detected between 2 and 8 species (Chandler, Hay and Reid, 1997; Klingen, Eilenberg and Meadow, 2002; Hummel *et al.*, 2002; Meyling and Eilenberg, 2006; Goble *et al.*, 2010; Clifton *et al.*, 2015; Uzman *et al.*, 2019). This low number of infected larvae can either be showing a low occurrence of fungi, or only a low detection. As we are only comparing soils within the same agroecosystem, we can only compare this figure to other studies also restricted to one site such as Meyling and Eilenberg (2006). In our study, on average 0.86 larva out of 10 in Kinsealy and 0.27 larva out of 10 in Nafferton were recorded as showing signs of fungal infection. Those figures are drastically lower than the 1.65 average from the Meyling and Eilenberg study. Entomopathogenic fungi either only occur seldomly in our systems or the low occurrence figures could also unfortunately be due to some artefacts of our method, such as poor conditions for sporulation on wet filter paper. Additionally, nematodes were ubiquitous in our samples. Even without being able to discriminate between general bacterial feeders and entomopathogenic nematodes, as nictating behaviour (when the nematode stands on its tail and waves its head in three dimensions) was observed in the vast majority of samples with nematodes presence, we can hypothesise than their occurrence was vastly greater than fungal infection. Research has shown the potential antagonism between EPF and EPN (Kaya et al., 1996; Shapiro-Ilan et al., 2004; Wu et al., 2014), specifically the antagonism between EPF and bacterial symbionts of EPN (Ansari et al., 2005). Only a limited number of studies include both types of pathogens in their baiting studies (Hummel *et al.*, 2002; Chandler and Davidson, 2005; Tkaczuk *et al.*, 2014) and their interactions and co-occurrence are not discussed. The great majority of our larvae appear to have died from bacterial pathogen infections, which are particularly effective against Lepidoptera (Lacey et al., 2015) such as *Galleria.* As such, this 3rd type of pathogen might also have competed with fungi and nematodes, or on the contrary acted synergistically with nematodes (Koppenhöfer et al., 1997). Bacterial entomopathogens are routinely used in industry, such as with *Bacillus thurengiensis* (Bt)

crops, and those pathogens have been shown to have great pest suppression potential (Kupferschmied et al., 2013; Lacey et al., 2001, 2015; Ruiu et al., 2013), however little is known about soil management impacts on those communities.

4.4.4 Taking into account variability over time in soil as well as mortality

Research using a similar baiting method to those reported in this thesis has shown the importance of replicating soil sampling over time and taking into account variability within and between years (Chandler, Hay and Reid, 1997; Hummel *et al.*, 2002; Meyling, Thorup-Kristensen and Eilenberg, 2011; Clifton *et al.*, 2015; Jaffuel *et al.*, 2016; Uzman *et al.*, 2019). Our summary tables (Table 38 and Table 41) and survival curves (Figure 59 and Figure 66) detailed the variability of differences between soils across sampling generations and years. However, perhaps the more striking difference over time is not in the expected variations between soil samples, but between survival rates at different points of the experiments. Kinsealy soils showed consistent suppression at 15D and 21D but Nafferton soils did not, as significantly higher mortality in conventional soils was found at 15D but not at the end of experiments. Apart from Meyling and Eilenberg (2006), who use time of death (in weeks) as a variable in a GLM, other studies only analysed larvae death at the end of the experiment (Goble et al., 2010; Hominick et al., 1990; Jaffuel et al., 2016; Koppenhöfer et al., 2006; Tkaczuk et al., 2014), even when mortality was recorded over time (Ingeborg Klingen et al., 2002; Tkaczuk et al., 2014). The aim of those studies differs from ours as soil baiting was not here only used as an entomopathogen occurrence survey, but as an estimation of overall potential for the soil to contribute to root pest mortality. Mortality rate over time can be drastically different when comparing a wider range of soils, as Chapter 5 will show, and we would argue that this variable can add precious information to characterise the soil suppression potential in a wider IPM context to reduce root herbivory quickly.

4.4.5 Limited answers provided by *Galleria* baiting

Any baiting methods will be selective (Meyling, 2007) and as a Lepidoptera, *Galleria* has been shown to be susceptible to different pathogens than Diptera *Delia* (I. Klingen et al., 2002). *Galleria* was used here as large numbers of age similar larvae can be procured quickly and cheaply and as this reliable method has been used repeatedly over the years. However, as our main aim was not the survey of specific pathogen species but the

evaluation of the soil suppression potential for root pest, baiting with *Delia* larvae would have provided more relevant results, more easily linked to our inoculation experiment and making the attempt at correlating baiting mortality and field pupae less dubious .This would have however required a substantially larger local *Delia* culture, carefully controlled to produce enough larvae of similar age. Retrospectively, this would have been a wiser use of our limited resources. If time had allowed, more effort should also have been invested in entomopathogen identification and complementary manipulation. Each dead larva should have been incubated individually (Uzman et al., 2019), especially to avoid nematode cross contamination and would have produced a greatly improved dataset. Additionally, soil solution plating (Kessler et al., 2003; Meyling, 2007; Meyling et al., 2006) would have helped characterised the fungi, whilst re-infecting larvae with baited nematodes, using Koch's postulate (Lacey, 2012), would have helped the confirmation of the insect pathogenic nature of the sampled nematodes, similarly to Jaffuel *et al.* (2016).

4.4.6 Including sterilised field soil as control

The use of sterile soil as a similar growing environment devoid of any entomopathogens or plant beneficial soil biodiversity should have been evaluated more carefully. This sterile control added information when included in our soil baiting, however, by autoclaving the soil, nutrients most likely were released in quantity into the soil (B.Griffiths, personal communication), leading to the confounding effects of the sterile aspect of the soil with the improved nutrient status of the soil. Across sites and replications, sterile plants were larger and pupae were not consistently positively impacted by the sterility of this soil, unlike the model pest during our soil baiting. Keeping the field soils used to produce our sterile control in order to grow plants in larger pots would have been a better use of our soil samples than the inclusion of a sterile control in the inoculation experiment, similar in texture but highly modified by the autoclaving process.

4.5 Conclusion

This chapter demonstrated that field soils can potentially contribute to pest suppression, when compared to their sterile counterparts. Similar to published work, our study failed to reveal significant difference of baited entomopathogens between soil managements. Detected entomopathogenic fungi occurrence and diversity was low and nematodes presence in most samples could not be accurately recorded as

entomopathogenic species or general bacterial feeders. Information provided by soil baiting was of limited relevance due to the selectivity of the lepidopteran pest and more resources should have been dedicated to entomopathogen identification, using *Delia* as a bait or at least a Dipteran bait. Using soil pairs across generations and years, we showed variations in model pest mortality and survival over time, reaffirming the need for replicated soil sampling and potential variability of pest suppression potential. Our complementary mortality analyses including survival analysis also showed that mortality assessment over the time of the experiment and not only at the end point in order can highlight important further variability. Soil management had an impact on plant growth in controlled conditions but the impact was not consistent across sites, pointing towards difference in soil functioning and characteristics between sites. Depending on the site, brassica plants allocated resources differently, which did not significantly impact pest survival. Inoculation reduced aboveground biomass by 10% or less but surprisingly did not consistently negatively impact the root system. No sign of improved bottom up control in organic soils was detected as inoculation had a similar effect on plants grown in the three different soils. Pupal numbers were only positively correlated to plant size and not negatively, potentially showing an unexpected impact of herbivory. As pupae count only gives a partial story on pest success and survival, damage score and pupal weight should also have been recorded. However, those limited results stem from the comparison of only two sets of experimental field soils. By using the same techniques but including a wider range of commercial soils and management practices, Chapter 5 will paint a contrasting picture regarding the pest suppression potential of soil microorganisms.

Chapter 5 Soil-based natural regulation: survey of paired commercial fields

5.1 Introduction

Previous chapters in this study have focussed on two experimental field sites, both including examples of organic and conventional practices. In Chapter 2 and 3, we demonstrated that management could have an impact on pest survival and natural enemy activity density, at plot level. Chapter 4 investigated the impacts of those contrasting managements on the microbial suppression potential of the soil as well as on pest-plant-soil interactions. Variability over time and between sites was important and our results of limited impacts as they were the fruit of the comparison of only two sets of contrasting practices. Whilst surveying a wider range of sites for the monitoring of local pest survival in parallel with its antagonist community would certainly not have been possible with limited resources and project timeframe, surveying soils of a wide range of brassica fields was however within our capabilities. This chapter reports results from a commercial soil survey, carried out in Autumn 2016 on 36 commercial farms across Great Britain and Ireland. Using a paired approach, organic and conventional field soils were compared to determine the impact of management on soil parameters as well as on the potential pest suppression service carried out by the microorganisms in the antagonist community.

As the impact of farming practices on the soil are wide ranging and organic management does not always lead to clear improvement (see Stolze et al., 2000), it was important for our study to specifically evaluate the impact of the practices included in this survey on the soil itself, at the time of sampling. Aside from helping understand our pest-soil systems, soil analyses across such a varied range of vegetable growing practices could also inform on the common contrasts between conventional and organic systems. This perhaps over-simplistic dichotomy has been criticized in various contexts (Le Campion et al., 2020; Lobley et al., 2009; Mander et al., 1999; Puech et al., 2014; Uzman et al., 2019; Winqvist et al., 2011) and as our study (at least its first three data chapters) is built around this opposition, this wider soil survey offered the opportunity to investigate further and test the validity of this dichotomy, in the context of pest regulation.

In parallel with soil analysis, methods adapted and used in Chapter 4 were used once again in order to assess the suppression potential of the sampled soils on the model pest with soil baiting, as well as on *Delia* exploring pest-plant-soil interactions with growth chamber inoculation experiments. As we could only include one soil sampling event per site, this prevented us from estimating the variability of the suppression potential over the growing season, which was highlighted in Chapter 4 as well as in other entomopathogenic studies using baiting techniques (Chandler et al., 1997; Clifton et al., 2015; Hummel et al., 2002; Jaffuel et al., 2016; Meyling et al., 2011; Uzman et al., 2019). With this limitation in mind, this survey still offers a valuable snapshot at one point in time of the impacts of varied managements on soil, pest suppression, entomopathogens presence, and impact on plant growth.

As soil functions and services are numerous and complex (Altieri et al., 2003; Altieri et al., 2005b; Birkhofer et al., 2008a; Ghaley et al., 2018; Roger-Estrade et al., 2010; Schulte et al., 2014; Tsiafouli et al., 2015), we used multivariate techniques in order to help identify links within the system, however without aiming to fully grasp the dynamics and processes at play. Using soils surveyed to investigate their potential root pest suppression without the mesofauna, whilst attempting to link this to soil parameters, can offer valuable insights on a facet of conservation biocontrol only seldomly studied.

Our research questions for this chapter are:

- How does conventional and organic management impact soil abiotic and biotic parameters?
- As practices are so varied, is the contrast of organic to conventional still valid?
- Does local pairing impact soil more than management or inversely?
- Does soil management impact model pest survival and occurrence of baited pathogens?
- Does organic management improve pest suppression in controlled conditions?

5.2 Material and methods

5.2.1 Soil survey set up

Our aim was to include at least 20 pairs of brassica fields, covering a wide geographical range as well as diverse soil types, local landscapes and growing conditions. Fields had to have been managed conventionally or organically for at least five years in

order to give enough time for management to impact the soil fully, and pairs needed to be located within 10 miles of each other and within the same landscape type.

In order to identify potential sites to survey, a simple Internet search was carried out, starting with organic vegetables growers (less numerous and more visible online). Also using personal contacts, SRUC staff and supervisor contacts, a list of both organic and conventional vegetable farms and cooperatives was created and owners and farm managers contacted via email or phone. If interested, they were asked if they could help identify a neighbouring organic or conventional brassica field that could constitute the second part of the pair. Thanks to the collaboration of farmers, farm managers and agronomists, 38 sites were chosen over several months. Prior to sampling, all sites were asked to give background information on the field selected (Annex 13), including pest and fertility management, crop rotations and brassica crop grown. The final 18 pairs of fields sampled are displayed on Figure 70, with some site details in Table 45. It should be noted that within one management category, farming practices and intensity varied greatly, as shown in Table 45.

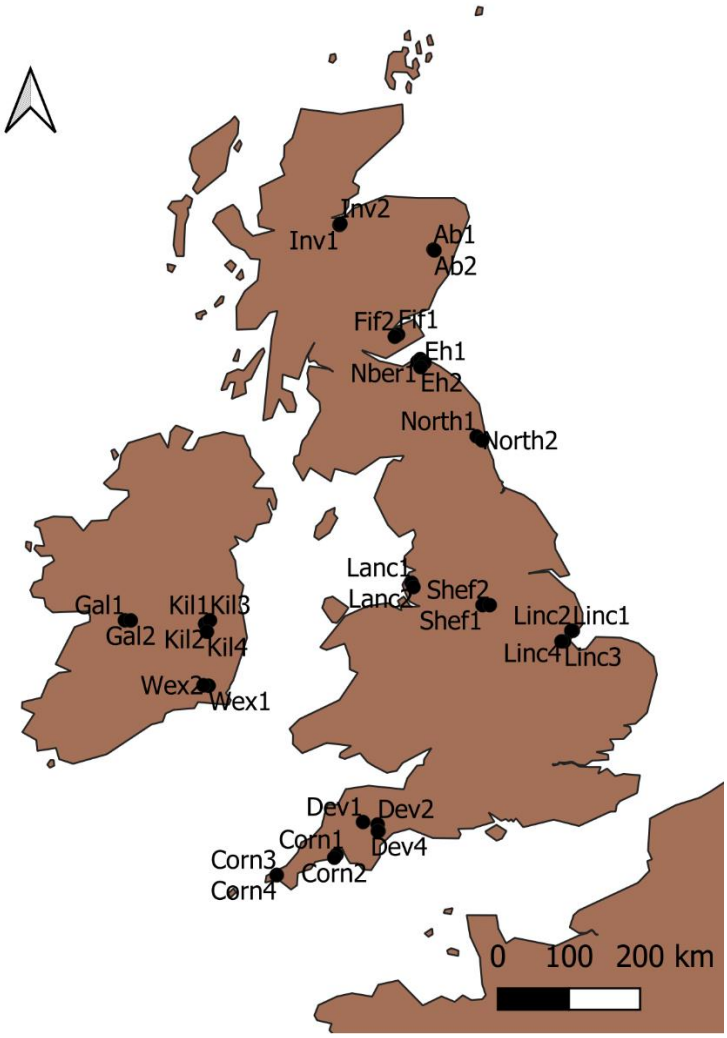


Figure 70 Soil survey sites with sample ID

Table 45 Organic and conventional sites sampled in Oct-Nov 2016 with sample ID

	Sample ID	Postcode	Farm type	Crop	Management	Soil type
conventional	Ab1	AB51 7LR	semi-intensive mixed arable and livestock	swedes	FYM, Spinosad	sandy loam
	Corn1	PL12 5BJ	Intensive large scale arable	winter OSR	netting, minimum tillage, intensive use of herbicide	sandy clay loam
	Corn3	TR27 5JQ	Intensive large scale vegetables	cauliflower	herbicide bleached the crop, double cropping	sandy clay loam
	Dev1	EX17 6DA	large scale but not intensive	swedes	no pesticides for 10 years, net and mesh together	sandy loam
	Dev3	EX6 7YL	mixed arable and livestock	stubble turnip	minimal use of pesticides	sandy clay loam
	Eh1	H41 3SJ	intensive arable	brussel sprouts	intensive use of pesticides	sandy clay loam
	Fif1	KY15 7UP	intensive large scale arable	broccoli (harvested)	double cropping	sand
	Gal1	Co. Galway, Ireland	intensive large scale arable	cabbage	net, chlorpyrifos drench	silty clay loam
	Inv1	IV2 6DL	mixed intensive arable and livestock	stubble turnip	intensive use of pesticides	sandy loam
	Kil1	Co. Kildare, Ireland	intensive large scale vegetables	brussel sprouts	Cyantraniliprole (Verimark) drench	sandy loam
	Kil3	Co. Kildare, Ireland	intensive large scale vegetables	broccoli (harvested)	Cyantraniliprole (Verimark) drench	sandy clay loam
	Lanc1	L40 8JL	intensive vegetables with ley crops	non headed cabbage	extensive use of green manure, Spinosad	sandy clay loam
	Linc1	PE22 0EJ	intensive vegetables	mustard	double cropping (broccoli before mustard), intensive use of pesticides	clay loam

	Linc3	PE22 9HE	intensive vegetables	cauliflower (2nd crop)	double cropping, Cyazypyr drench and Spinosad, intensive use of pesticides	silty clay
	Nber1	EH39 5AT	intensive arable	brussel sprouts	FYM, mushroom compost, intensive use of pesticides, chlorpyrifos drench	sandy silt loam
	North1	NE22 6A2	intensive arable	OSR	intensive use of pesticides	clay
	Shef1	S8 8BG	mixed intensive arable and livestock	OSR	pig slurry in Autumn, minimum tillage, medium use of pesticides	sandy clay loam
	Wex1	Co. Wexford, Ireland	semi-intensive arable	cabbage	net, chlorpyrifos drench, intensive use of pesticide	sandy loam
organic	Ab2	AB51 7LS	small scale	mixed brassica	green manure, FYM, Spinosad, net	sandy clay loam
	Corn2	PL11 3DJ	medium scale	kale and cabbage	no pesticide, net	silty clay loam
	Corn4	TR27 5JQ	large scale	cauliflower	FYM	silty loam
	Dev2	EX5 5HY	large scale	mixed brassica	municipal green waste, 2 years clover ley	sandy loam
	Dev4	EX2 9QQ	small scale	mixed brassica	municipal compost, chicken manure, plugs drenched in compost tea	sandy loam
	Eh2	EH34 5BD	medium scale, biodynamic	swedes	biodynamic, FYM, clover ley	silty clay loam
	Fif2	KY15 7AD	small scale	savoy cabbage	green manure, 2 years clover and rye, net	sandy silt loam
	Gal2	Co. Galway, Ireland	medium scale	cabbage	green manure, net, delayed planting	silty loam
	Inv2	IV2 6DJ	small scale market garden vegetable	mixed brassica	FYM, net, 2 years clovers and rye	silty loam
	Kil2	Co. Kildare, Ireland	large scale	brussel sprouts	organic poultry manure, net	sandy clay loam
	Kil4	Co. Kildare, Ireland	large scale	kale	net, Spinosad, green manure	silty clay loam
	Lanc2	L39 0EE	large scale	cabbage	FYM, sea weed foliar spray, Spinosad	loamy sand
	Linc2	PE20 1JD	large scale	broccoli	FYM, net	silty clay
	Linc4	PE22 9BT	mixed large scale	broccoli	FYM, Spinosad, 2 years clover	silty clay
	Nber2	EH39 5LP	large scale	cabbage	Bt, 2 years grass	sandy clay loam

	North2	NE22 7AD	small scale market garden vegetable	broccoli and brussel sprouts	6 months composted FYM, net, green manure	silty clay
	Shef2	S8 8BG	small scale market garden vegetable	mixed brassica	leaf mould, chicken pellets, horse and FYM	clay loam
	Wex2	Co. Wexford, Ireland	medium scale	cabbage and brussel sprouts	compost, net	sandy loam

Soils were sampled in October-November 2016 by the author on UK farms and by Leo Finn on Irish farms. Sampling was carried out across the whole field using a W pattern to produce five replicates, away from field edges and avoiding compacted areas. Using a trowel and zip bag, around 2 kg of soil was sampled per GPS location from the first 15 cm soil depth. Once back at the laboratory, all samples were stored at 4°C awaiting processing.

5.2.2 Soil analysis and pest experiments

Soil analysis

Each bagged soil sample was examined and all visible mesofauna was removed using stork bill forceps. All earthworms were extracted and counted, recording data per bagged sample, adjusting for an average sample weight of 2 kg. Soil samples were then mixed at field level. From those composite samples, 250 g was reserved for pathogenic free-living nematodes extraction and another 200 g for soil analysis. Soil texture was determined by hand with the help of Dr Joanna Cloy and Dr Bruce Ball. Free-living nematode extraction and identification were carried by Dr Roy Nielson (James Hutton Institute). Earthworm count and CLPP were determined by the author. All other soil parameters were determined by John Parker and Maria Stanisz-Migal.

All soil parameters measured are listed below in Table 46 and complete protocols are included in annexes specified.

Table 46 Measured soil parameters labels, definitions and reference to method used

	Label (units)	Definition	Method
Abiotic parameters	%DM	Percentage of dry matter: informs on soil water content at the time of analysis. Used to rescale other parameters.	SOP Soil dry weight Annex 01
	DOC (µg C/g soil DM)	Dissolved organic carbon: product of decomposition of litter and humus but could also originate directly from exudates from plant roots. It is usually operationally defined as the organic carbon which can pass through a 0.45 µm filter (van den Berg et al., 2012)	SOP Microbial Biomass carbon – 1st part DOC in unfumigated soil Annex 10
	HWEC(µg C/g soil DM)	Hot water extracted carbon: component of the labile SOM, closely related to soil microbial biomass and microaggregation. It is strongly correlated with CO_2 evolution which would indicate that a proportion of the HWC must be easily available for	SOP HWC adapted from Ghani et al., 2003 Annex 06

		microbial utilisation (Ghani et al., 2003)	
	Mineral NH_4 N (µg/g soil DM)	Mineral nitrogen from NH_4	SOP KCl Extract of Soils for NH_4-N and NO_3-N, then analysed using a continuous flow analyser (Skalar San++ 4800, Netherlands) Annex 07
	Mineral NO_3 N (µg/g soil DM)	Mineral nitrogen from NH_3	SOP KCl Extract of Soils for NH_4-N and NO_3-N, then analysed using a continuous flow analyser (Skalar San++ 4800, Netherlands) Annex 07
	pH	Soil pH	SOP Measurement of soil pH in fresh soil Annex 02
	%LOI	Percentage of loss on ignition – measure of soil organic matter	SOP LOI Annex 08
Biotic parameters	Mineralisable NH_4 N (µg PMN NH4:N per g soil DM)	Conversion of organic N into mineral forms available to plants, which takes place through the biochemical transformation mediated by microorganisms (Stevenson, 1985).	SOP PMN, adapted from from Canali and Benedetti, 2006 Annex 09
	Biomass C (µg/g soil DM)	Amount of carbon from soil microbial organisms measured after soil fumigation – proxy for microorganism abundance	SOP Microbial Biomass carbon Annex 10
	Biomass N (µg/g soil DM)	Amount of nitrogen from soil microbial organisms measured after soil fumigation - proxy for microorganism abundance	SOP Microbial Biomass carbon, with total dissolved N analysed using a continuous flow analyser (Skalar San++ 4800, Netherlands) Annex 10
	AWCDt5 (absorbance at 595nm)	Average well colour development at 5 days at 595 nm for Ecoplate Community Physiological Profile - proxy for microbiological activity.	SOP CLPP Ecoplates Annex 04
	FLN count (per 200g of soil)	Pathogenic free-living nematodes count	SOP Free living nematodes Annex 05
	Earthworm count (per 2kg of soil)	Earthworms extracted from soil samples	As described above

Soil baiting

Soil baiting was carried out using the same method described in Chapter 4 using five soil tub replicates per soil, with one major difference: as soil quantity was very limited, no sterile control was included in this baiting set. Mortality was recorded over time at 3 days interval, and analysed at 10 days and 19 days. All experiments were carried out for 25 days, at the end of which presence of nematodes and sporulating fungi were recorded.

Inoculation experiment

Pest-plant-soil interactions were also explored with an inoculation experiment using the same method described in Chapter 4, with some adaptations. Firstly, as soil quantity was limited, no sterile control was included and only one variety (Belstar) was used. Secondly, 1.5 L pots were used instead of the 11 cm pots used in Chapter 4, to allow the plant better root development and give more space for larvae to develop into pupae. Thirdly, as soil quantity left after the soil baiting experiment was variable across sites, the number of replicates per site varied between 2 (only Gal2) and 5 and led to an unbalanced experimental design. And lastly, this time, herbivory damage was scored on freshly washed root systems (Figure 72), following Chapter 2's scoring system, shown again in Table 47.

Table 47 Stem damage scoring system (Hopkins, 1994)

Score	Criteria
0	undamaged
1	less than 25% of root area damaged
2	25-50% of root area damaged
3	more than 50% of root area damaged
4	more than 75% of root area damaged/severely destroyed

.

Figure 71 Inoculation experiment in growth chamber conditions with soil survey soils, showing the larger 1.5L pots

Figure 72 Example of washed root systems at the end of the soil survey inoculation experiment at the moment of damage scoring (inoculated root systems with orange labels)

The great majority of broccoli developed well and survived, with fewer aphid issues in the growth room, as well as fewer obvious nutrition deficiencies. Two of the soils sampled, Fif1 and Ab1, contained clubroot (*Plasmodiophora brassicae*), as shown in Figure 73, which greatly reduced plant fitness.

Figure 73 Example of washed root systems at the end of the soil survey inoculation experiment, showing signs of clubroot (*Plasmodiophora brassicae*)

5.2.3 Statistical analysis

To help answer our first research question on management impacts, all soil quantitative parameters measured were first analysed using a simple GLM, using management as fixed model and pair as random model. All soil parameters were then used in a correlation (Pearson coefficient) to build a better picture of possible links within the multivariate dataset. To answer our second research question on the validity of organic and conventional labels, a forward selection discriminant analysis (linear method) was used, setting the number of optimal variables to 10, 7 or 5 in order to compare reclassification errors. To investigate our third question, on the relative impact of pairing and management, a cluster analysis was carried out using the nearest neighbour method.

Soil baiting results were analysed as in Chapter 4, using mortality at 10 days and 19 days in a GLMM Binomial analysis to investigate the impact of management, as well as using the survival data over time to produce the survival function (Kaplan Meier estimate). Presence of entomopathogens was analysed similarly to Chapter 4 using a GLMM Poisson, and both mortalities and entomopathogens presence were used in a correlation (Pearson coefficient) to highlight any potential link between rate of mortality over time and type of pathogen present. Inoculation experiment variables were also analysed as in Chapter 4, with damage analysed with a GLMM Poisson distribution (score data). Discriminant analysis was used again including pest experiment variables, in order to test any possible improvement on the model when including those extra parameters that could be included in further soil health and function comparisons.

5.3 Results

The first part of the result section contains the univariate and multivariate analysis of soil parameters. The second part contains the pest experiment variable analyses, followed by the overall analysis including all variables.

5.3.1 Overall soil characterisation

Single parameter approach: impact of management on soil as habitat

Results are presented separately for abiotic parameters (Table 48) and biotic soil parameters (Table 49) for clarity.

Table 48 Abiotic factor analysis comparing organic and conventional soils surveyed (significant differences are in bold)

Abiotic parameters	conventional (mean ± SEM)	organic (mean ± SEM)	GLM output
%LOI	**4.94±0.32**	**5.87±0.42**	**F=5.68, p=0.029**
%DM	80.10±0.95	78.79±1.15	F=2.7, p=0.119
DOC (µg C/g soil DM)	47.48±6.24	48.86±6.31	F=0.04, p=0.835
HWEC(µg C/g soil DM)	665.6±49.21	740.9±51.84	F=2.51, p=0.132
Mineral NH_4 N (µg/g soil DM)	0.63±0.17	0.77±0.17	F=0.61, p=0.447
Mineral NO_3 N (µg/g soil DM)	26.78±6.17	21.90±1.92	F=0.6, p=0.45
pH	6.71±0.22	6.80±0.16	F=0.22, p=0.647

Analysis of the abiotic parameters only showed one significant difference: loss on ignition was significantly higher in organic soils. It is worth pointing out that mineral NO_3 N was not significantly higher in conventional soils despite the application of mineral fertiliser. Moreover, despite a higher loss on ignition for organic soils, no significant difference was found between soils for dissolved organic carbon and hot water extracted carbon, even with a 10% larger value for the latter for organic soils.

Table 49 Biotic factor analysis comparing organic and conventional soils surveyed (significant differences are in bold)

Biotic parameters	conventional (mean ± SEM)	organic (mean ± SEM)	GLM output
AWCDt5 (absorbance at 595nm)	**0.92±0.04**	**1.08±0.04**	**F=12.79, p=0.002**
Biomass C (µg of biomass C/g of soil DM)	399.6±39.34	446.9±35.05	F=1.5, p=0.237
Biomass N (µg of biomass N/g of soil DM)	15.06±3.17	15.66±1.92	F=0.05, p=0.834
Mineralisable NH_4 N (µg PMN NH_4:N /g soil DM)	**31.89±4.37**	**40.31±3.57**	**F=4.87, p=0.042**
Pathogenic FLN count (per 200g of soil)	**117.72±20.49**	**74.89±9.25**	**F=5.34, p=0.034**
Earthworm count (per 2kg of soil)	**1.34±.36**	**4.44±0.73**	**F=13.94, p=0.002**

Microbial activity as measured by CLPP AWCDt5 was significantly higher in organic soils. Biomass C mean was higher in organic soils, but not significantly so compared to conventional soil, whilst biomass N means were very close for both soils. Mineralizable NH_4 N was also significantly higher in organic soils. Earthworms count was significantly higher in organic soils whilst pathogenic free-living nematodes count was lower. Even without a significant increase in all carbon sources in organic soils, biological activity was overall higher in organic soils sampled, across different taxa. In terms of function, this points towards a higher turn-over in the 15 top cm of organic soils sampled both at microbial scale for nutrients and macro scale through earthworm activity (B.Griffiths, pers.comm.).

Multivariate approach: correlation between soil parameters

Before going further in our multivariate analysis, all quantitative soil parameters measured were included in a correlation, in order to identify links within the soil dataset and especially highlight the parameters not correlated with any other parameter, thus adding new information to the multidimensional dataset. As 90 pairs were being tested, a Bonferroni correction was needed, with a new alpha level of 5.5.10-4. As Genstat only reports p values below 0.001 as <0.001 with no specific value, we unfortunately cannot correctly reach the adjusted alpha level and can only report correlations significant at $p<0.001$.

Table 50 Soil parameters correlation- Pearson coefficient (p value), non significant in light grey

	%_DM	%_LOI	AWCDt5	BiomassC	BiomassN	DOC	FLN_count	HWEC	Mineral_NH_4_N	Mineral_NO_3_N	Mineralisable_NH_4_N	Worm Average
%_DM	-											
%_LOI	-0.666 (<0.001)	-										
AWCDt5	-0.207	0.058	-									
BiomassC	-0.5134 (<0.001)	0.293	0.164	-								
BiomassN	-0.5432 (<0.001)	0.293	0.3088	0.277	-							
DOC	-0.265	0.078	-0.045	-0.229	0.245	-						
FLN_count	0.210	-0.021	-0.202	-0.119	-0.296	-0.108						
HWEC	-0.6485 (<0.001)	0.54845 (<0.001)	0.163	0.4554	0.4503	0.142	-0.136	-				
Mineral_NH4_N	-0.3132	0.3656	-0.011	0.282	0.275	0.131	-0.068	0.116	-			
Mineral_NO3_N	-0.117	0.211	-0.015	0.291	-0.145	0.011	0.089	0.289	0.091	-		
Mineralisable_NH4_N	-0.6775 (<0.001)	0.7815 (<0.001)	0.099	0.3862	0.4096	0.188	-0.081	0.5136	0.56115 (<0.001)	0.082	-	
WormAverage	-0.246	0.308	0.3948	-0.014	0.114	0.037	-0.3189	0.216	-0.038	0.207	0.205	-
pH	0.120	-0.276	0.243	-0.202	-0.077	0.105	-0.205	-0.199	-0.5278 (<0.001)	-0.198	-0.3251	0.226

As this correlation only comes from one sampling event in Autumn 2016, this can only represent a snapshot of soil health and functions at the time of sampling. The Bonferroni correction also reduced greatly the number of significant correlations. However, a few links are still worth highlighting.

Carbon sources

Soil organic matter as measured with % loss on ignition was positively correlated to hot water extracted carbon but surprisingly not to dissolved organic carbon, the product of decomposition of litter and humus. It was also correlated with mineralizable NH_4 N.

Nitrogen

It is worth noting that NH_3 N was not correlated with any other variable. Unlike NH_4 N and mineralizable NH_4 N, this form of nitrogen was not linked to any other variables, showing its particular status in our dataset, as adding extra information that no other variable can add.

Biological activity across taxa

Perhaps surprisingly, after the correction, no biotic parameters were correlated, apart from biomass C and N negatively correlated to % dry matter, showing the limited relevance of a simple correlation in order to frame soil functioning.

Multivariate approach: organic VS conventional

As practices vary widely within the organic and conventional categories, this simple dichotomy might not appear relevant when considering soil based natural regulation, similarly to entomopathogens in Chapter 4. As a first attempt to determine if those labels should still be used here, both abiotic and biotic soil parameters were included in a stepwise discriminant analysis with forward selection. This analysis determines if this simple soil dichotomy holds in re-sorting soils (using bootstrapping) within those two categories after creating a model using all parameters measured. It also highlights which soil parameters are more relevant to make this distinction for the dataset considered. Results are shown below (Figure 74 and Figure 75).

Optimal variables	Counts	management		
WormAverage		conventional	organic	Total
Mineral_NO3_N	allocated			
BiomassC	conventional	16	.	16
BiomassN	organic	2	17	19
AWCDt5	Total	18	17	35
DOC	Dev1 misclassified as organic			
pH	Shef1 misclassified as organic			
%_DM				
% LOI				

Using bootstrapping		
with 632 rule to calculate errors		
Error: 16.53%		
Percentage of each group allocated to groups		
	True group	
Decision	conventional	organic
conventional	76.74	9.61
organic	23.26	90.39

Figure 74 Discriminant analysis using 10 variables for all soil sampled except Shef2 (no mineralizable NH4 N value), using forward selection, showing reclassification and errors

Optimal variables	Counts	management		
WormAverage		conventional	organic	Total
Mineral NO3 N	allocated			
	conventional	16	3	19
	organic	2	14	16
	Total	18	17	35
	Gal1	misclassified as organic		
	Gal2	misclassified as conventional		
	Kil2	misclassified as conventional		
	Linc1	misclassified as organic		
	Nber2	misclassified as conventional		

Using bootstrapping		
with 632 rule to calculate errors		
Error: 17.57%		
Percentage of each group allocated to groups		
	True group	
Decision	conventional	organic
conventional	85.67	21.14
organic	14.33	78.86

Figure 75 Discriminant analysis using 5 variables for all soil sampled except Shef2 (no mineralizable NH4 4), using forward selection, showing reclassification and errors

The model created by both discriminant analyses could indeed sort out organic and conventional samples using soil parameters with 17% error average. Dev1 and Shef1 samples were both misclassified as organic samples with the 10 variables discriminant, with more classification errors for the 5 variables discriminant. It should be noted that all biotic soil parameters apart from mineralizable NH_4 N are part of the 10 variables optimal set, even without the presence of significant differences in the GLMs. Out of the top five variables, the only abiotic parameter is the NO_3 N, which was not correlated with any other variable and not significantly higher in conventional soils despite a higher value and the use of mineral fertilisation in conventional soils. When only using five variables to discriminate between soils, the model only required two variables, namely worm average and mineral NO_3 N. Reducing the number of optimal variables to two only led to a slight increase in reclassification error, from 16.53 to 17.57%.

Multivariate approach: pair and management cluster

In order to answer our third research question and determine if physical pairing within the landscape had a stronger impact than soil management on soil parameters, all soils sampled were included in a cluster analysis, using the nearest neighbour method. All odd sample IDs represent conventional soils, and all even sample IDs represent organic soils.

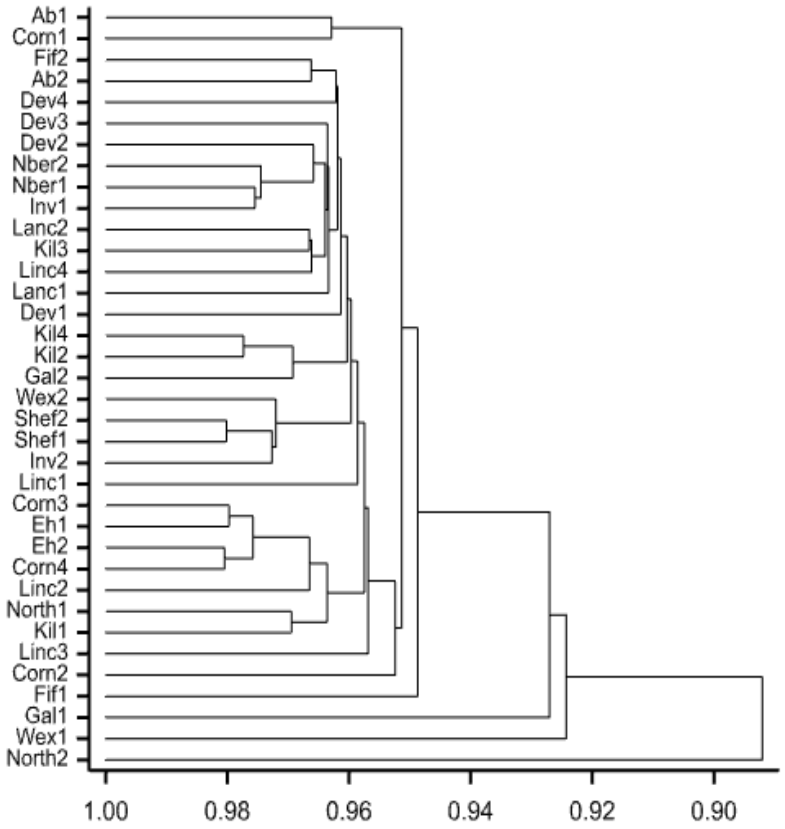


Figure 76 Cluster analysis (nearest neighbour) including all soils parameters, with similarity index as x axis

Physical pairing did not have a strong influence on soil parameters as out of 18 pairs, only four were most similar and neighbours on the dendrogram: Dev3 and 4, Nber1 and 2, Shef1 and 2 and Eh1 and 2. Those organic and conventional paired soils were still different enough however not to be misclassified in the previous DAs. Considering the overall similarity index on the x axis, the most dissimilar soils were North2, Wex1 and Gal1, which might then be expected to be the source of outliers for our other experiments. On

closer inspection, North2 had the highest LOI at over 10% (mean organic 5.87±0.42%), the highest mean number of worms at 13 (mean organic 4.44±0.73) and the highest HWEC at 1143 µg $C.g^{-1}$ soil DM (mean organic 740.9±51.84 µg $C.g^{-1}$ soil DM). Wex 1 had the highest mineral NO_3 N at 96.68 µg.g^{-1} soil DM (mean conventional 26.78±6.17 µg.g^{-1} soil DM) and Gal 1 had the highest biomass N at 66.02 µg of biomass N.g^{-1} of soil DM (mean conventional 15.06±3.17 µg of biomass N.g^{-1} of soil DM).

5.3.2 Soil baiting

Overall analysis conventional and organic (GLMM mortality at 10D and 19D)

Firstly, overall analysis comparing organic and conventional management is presented here, including both mortality of model pest at 10 days and 19 days.

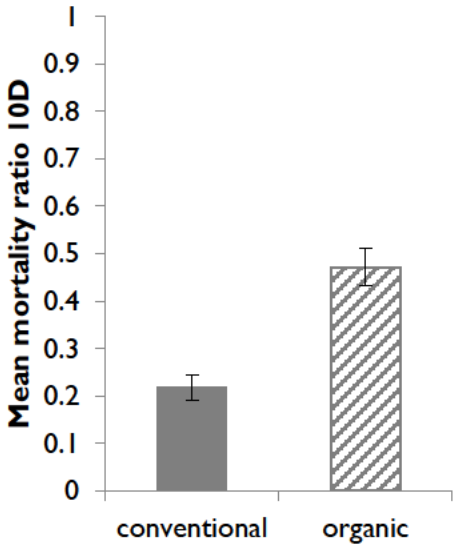


Figure 77 Overall mortality ratio at 10 days (±SEM)

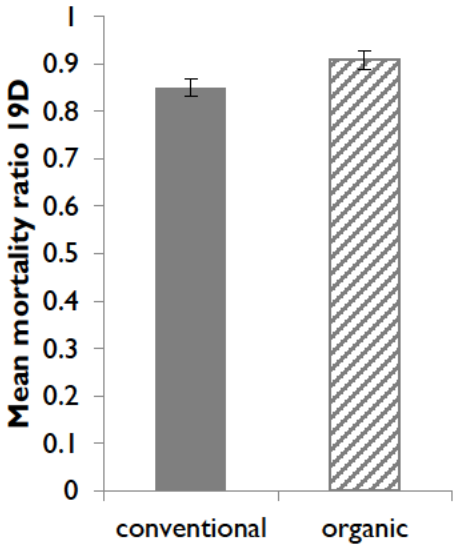


Figure 78 Overall mortality ratio at 19 days (±SEM)

Overall analysis of the mortality ratio of the model pest from the 38 soils showed a significant difference in mortality after 10 days (Figure 77) , with higher mortality in organic soils (GLMM Binomial, F=4.85, df=31.07, p=0.035, conv=0.22±0.02 and org=0.47±0.04). However, after 19 days (Figure 78), the gap closed and the significant difference at 5% disappeared (GLMM Binomial, F=3.37, df=31.7, p=0.076).

Paired approach (survival analysis and paired t-test)

In parallel to the overall management comparison, soil samples were also analysed as pairs, first using the survival analysis method, similar to Chapter 4 (Kaplan Meier

estimate) coupled with a paired t-test, comparing mortality at 10D and 19D within a pair, as non-parametric testing of those estimates was not available with GenStat.

Survival analysis by pairs yielded varied responses, summarised in Table 51 as four main scenarios. Figure 79 and Figure 80 display examples of the first scenario, where no difference was found between organic and conventional soil, resulting in either a high overall mortality (Figure 79) or a low mortality (Figure 80). Figure 81 and Figure 82 show examples of the second scenario with higher mortality in organic soils, where survival in organic soil either dipped very quickly (Figure 81) or stayed similar to conventional soil before accelerating (Figure 82). The reverse scenario is illustrated by Figure 83 where organic survival dropped quickly early on, to then be caught up by conventional mortality, slowly increasing over time. Figure 84 shows a similar mortality rate in both soil at 10 days, with organic mortality accelerating towards the end of the experiment. Finally, Figure 85 and Figure 86 illustrate the last, less common, result where conventional mortality was higher than organic mortality. Conventional mortality was higher than organic mortality only either at 10 days (Figure 85) or 19 days (Figure 86), but never for both. Overall, out of 18 pairs, four showed no difference, 14 showed a higher mortality in organic soil for at least part of the experiment, and four pairs showed a higher mortality in conventional soil. None displayed a switch in pattern between mortality at 10D and 19D (Table 51).

Table 51 Soil samples pairs survival analysis summary, using paired t tests to discriminate between pair behaviour

Outcomes	Time points	Soil pairs	Total
No difference in mortality	Kil1+2, Lanc, Linc3+4, North		4/18 pairs
Higher mortality in organic soil	both at 10 and 19 days	Corn1+2, Gal, Inv, Kil3+4, Wex	10/18 pairs
	at 10 days	Eh, Fif, Nber	
	at 19 days	Dev1+2, Dev3+4	
Higher mortality in conventional soil	both at 10 and 19 days	none	4/18 pairs
	at 10 days	Ab, Linc1+2	
	at 19 days	Shef, Corn3+4	
Switch in pattern between 10 and 19 days	none		0/18 pairs

Full t-tests results and survival curves in annex 14.

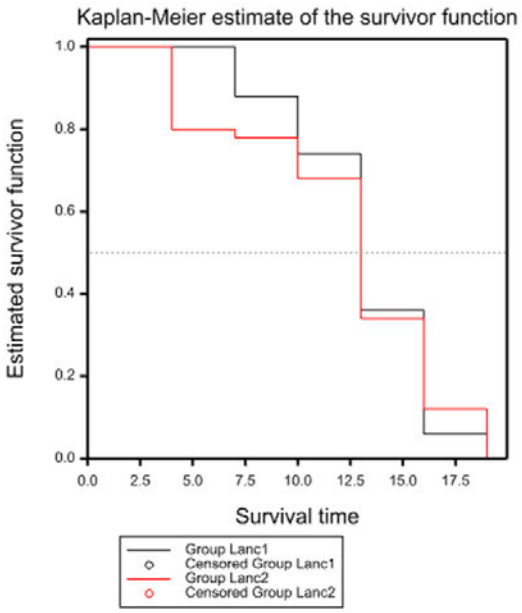


Figure 79 Survival analysis for Lanc1 and Lanc2, showing similar model pest survival curve, with high mortality

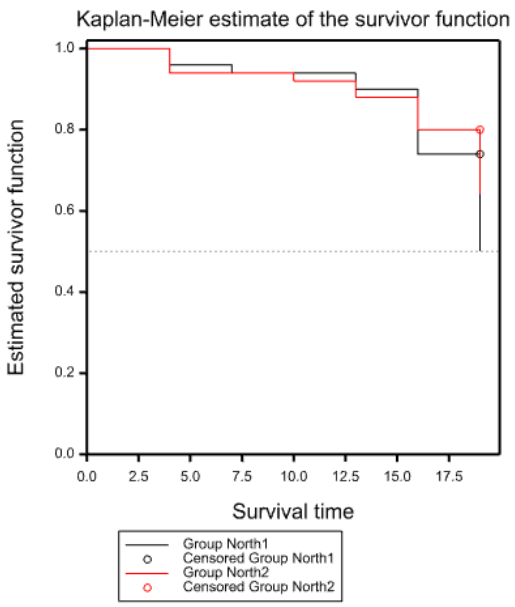


Figure 80 Survival analysis for North1 and North2, showing similar model pest survival with low mortality

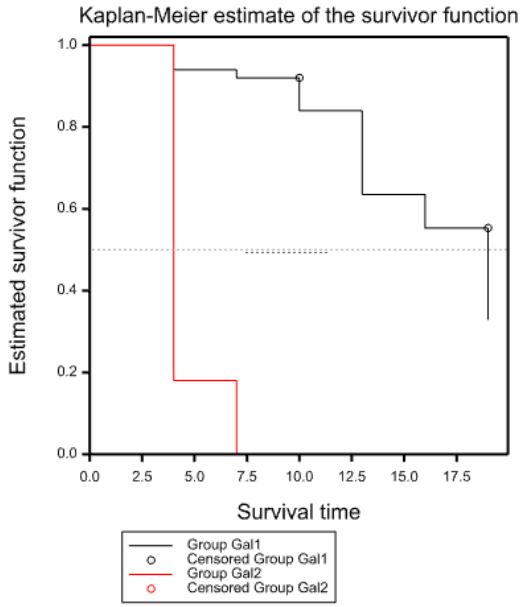


Figure 81 Survival analysis for Gal1 and Gal2, showing rapid mortality in Gal2

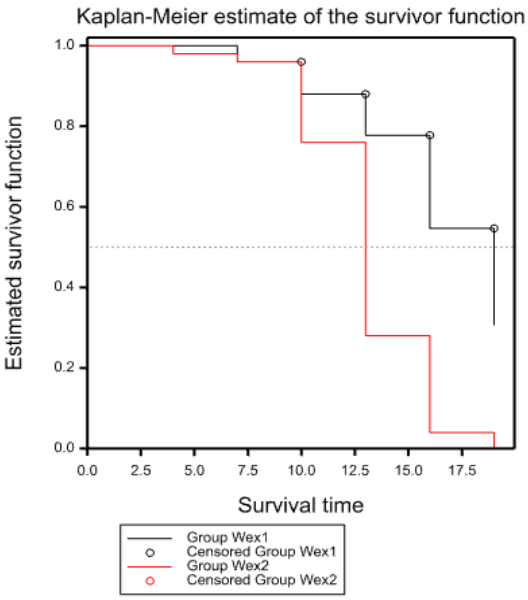


Figure 82 Survival analysis for Wex1 and Wex2, showing a slower mortality rate in Wex2

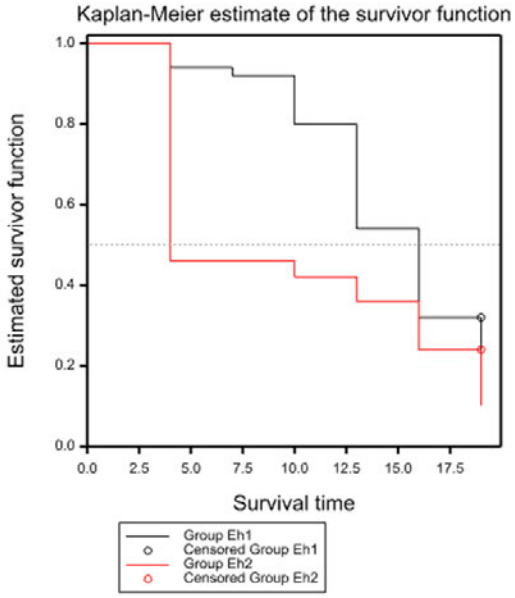


Figure 83 Survival analysis for Eh1 and Eh2, showing a higher mortality for Eh2 at 10 days followed by no difference at 19 days

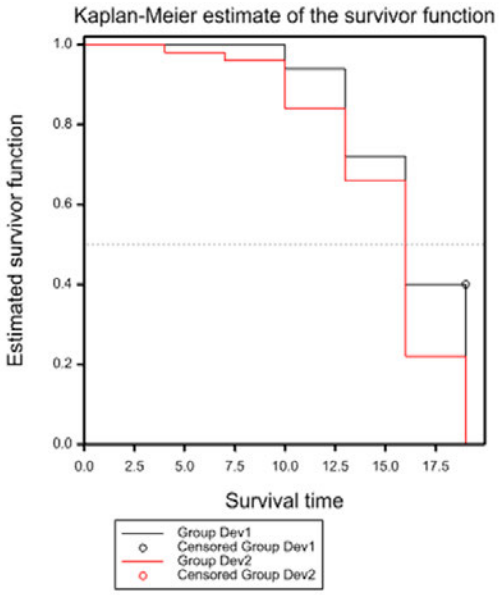


Figure 84 Survival analysis for Dev1 and Dev2, showing no difference at 10 days, followed by a higher mortality for Dev2 at 19 days

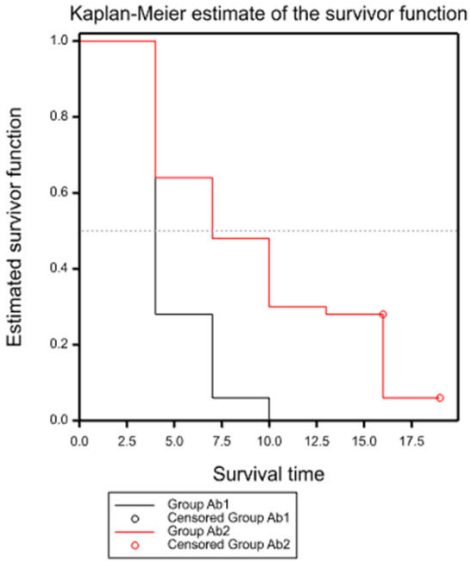


Figure 85 Survival analysis for Ab1 and Ab2 showing a higher mortality for Ab1 for 10 days followed by no difference in mortality at 19 days

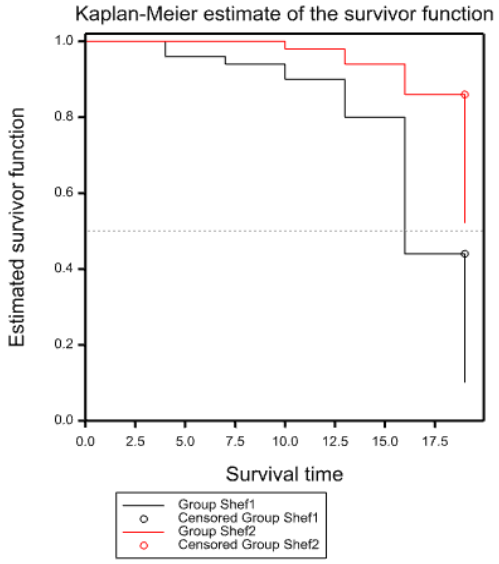


Figure 86 Survival analysis for Shef1 and Shef2 showing no difference in mortality at 10 days followed by a higher mortality for Linc1 at 19 days

Presence of entomopathogens

Nematodes were found on dead larvae in at least one soil tub out of five from all soils. As dead larvae extracted from the same soil tub were placed together in the same Petri dish and nematodes could have easily travelled from one larva to the next on the wet filter paper, no individual infected larvae count was possible and only presence/absence of nematodes in the subsample could be recorded. The binary data was analysed using a GLMM Binomial that did not produce a significant result at 5% (GLMM Binomial, F=3.63, df=30.8, p=0.066). Results are shown on Figure 87.

In order to produce comparable data, sporulating fungi was also recorded as presence/absence in replicate tub, and analysed with GLMM Binomial. Again, the mean presence in organic soil was higher, but not significantly so (GLMM Binomial F=1.08, df=33.2, p=0.31). Results are shown on Figure 88.

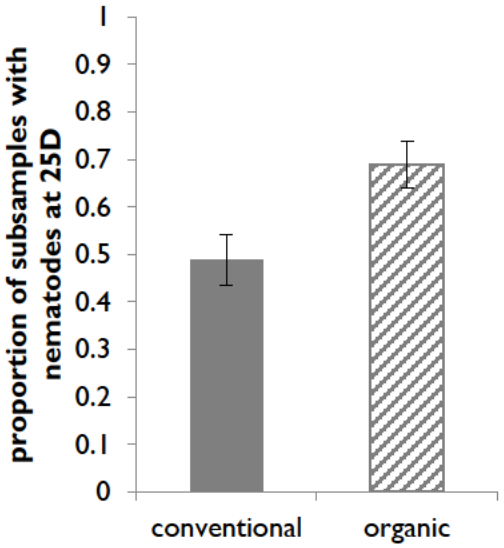


Figure 87 Mean proportion (±SEM) of soil tubs with nematodes at 25 days

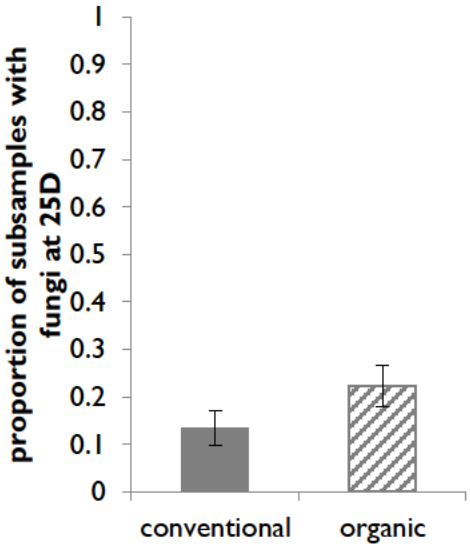


Figure 88 Mean proportion (±SEM) of soil tubs with sporulating fungi at 25 days

Linking mortality to presence of entomopathogens

A correlation (Pearson coefficient) was used in order to investigate a possible link between mortality at 10 and 19 days with the presence of entomopathogens. Results are shown as a heat map in Figure 89. Mortalities at 10 days and 19 days were positively correlated at only 46%, showing the need for the measure of mortality over time, and not at a single point. Mortality at 10 days was positively correlated with the presence of fungi at

16% and the presence of nematodes at 14%. Mortality at 19 days was almost positively correlated to the presence of sporulating fungi (p=0.06) but not correlated at all to the presence of nematodes. Presence of nematodes and sporulating fungi were negatively correlated at -15%.

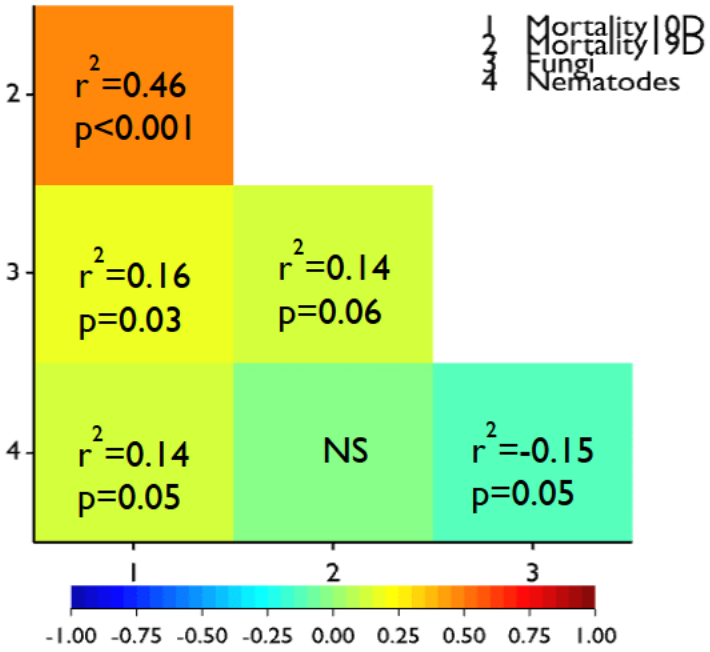


Figure 89 Correlation map between mortality at 10 days, 19 days and presence of nematodes and fungi at 25 days

5.3.3 Inoculation experiment: plant growth and pest survival in sampled soils

Firstly, single parameter analyses for each variable measured are presented, followed by multivariate approach.

Aboveground biomass

Measurements of aboveground biomass of *B. oleracera* contained a few large outliers so the log transform was used for the GLM, after extreme outliers were removed (Kil1.3=30.31g, Gal1.3=22.7g). Neither inoculation nor management had a significant impact on aboveground biomass, as shown in Table 52. It is worth noting that the top part of the organic plants was not significantly smaller compared to conventional plants.

Table 52 Aboveground biomass GLMs

factor	method	means(±SEM)	F ratio	df	p value
management	GLM	org=6.34±0.30, conv=5.98±0.25	1.03	36.4	0.317
inoculation	GLM	eggs=6.06±0.27, no_eggs=6.27±0.29	1.23	219.8	0.269

Belowground biomass

Log transform for belowground biomass was also used for the GLM because of large outliers, after the same extreme outliers were removed (Kil1.3=11.08g, Gal1.3=9.96g). Contrasting with aboveground biomass, both management and inoculation had a significant impact on belowground biomass, as shown in Table 53. Organic and conventional plants reacted in a similar way to inoculation as no significant interaction was found between factors. Organic plants had a significantly larger root system and inoculated plants had a significantly smaller root system.

Table 53 Belowground biomass GLMs

factor	method	means(±SEM)	F ratio	df	p value
management	GLM	org=2.62±0.13, conv=2.27±0.11	4.41	36.4	0.043
inoculation	GLM	eggs=2.33±0.12, no_eggs=2.58±0.13	5.18	219	0.024

Recovered pupae

The first GLMM Binomial was run on all inoculated plant pupae counts, using inoculated egg numbers as binomial totals. No significant difference was found between managements (GLMM Binomial, F=0.19, df=39.6, p=0.663; org=0.65±0.14, conv=1.43±0.44). Similar to Chapter 4, pupae counts contained numerous zeros. The second GLMM Binomial was run on a further restricted dataset, only including non-zero values. When pupae were present, management did have a significant effect on pest survival and 63% fewer pupae were found in organic root systems (GLMM Binomial, F=7.25, df=24.7, p=0.013; org=1.83±0.24, conv=4.9±1.19).

Root damage

GLMM Poisson was run on root damage score on inoculated plants. Both management and pupal numbers were included as factors. Management did not significantly impact damage score, however pupal numbers did, as shown in Table 54.

Table 54 Root damage GLMs

factor	method	means(±SEM)	F ratio	df	p value
management	GLMM Poisson	org=2.53±0.13, conv=2.71±0.11	2.73	35.4	0.107
pupae number	GLMM Poisson	-	10	128.4	0.002

Link between measurements

A correlation (Pearson coefficient) was used in order to investigate a possible link with the plant-pest system (Figure 90).

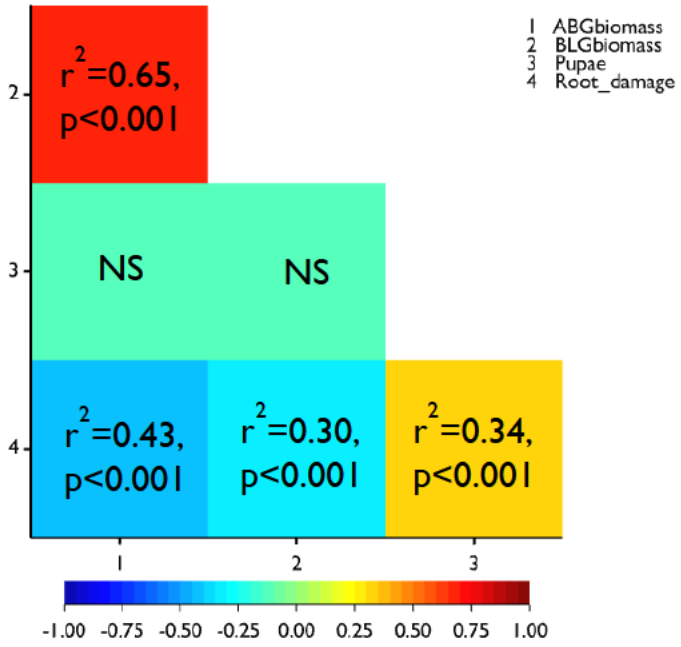


Figure 90 Correlation between plant and pest parameters

Root damage score was significantly positively correlated to aboveground biomass (43%), belowground biomass (30%) as well as pupae count (34%). Aboveground and belowground biomasses were significantly positively correlated at 65%. The potential effect of management on the link between root damage and pupae count could not be further investigated reliably as pupae count data had numerous zeros and root damage score being

a discreet scale measurement of 5 possible scores. Instead of using a root to shoot ratio analysis, a further regression was run in order to test if plants invested resources differently depending on soil management and inoculation. Inoculation did not have a significant impact on the aboveground biomass – belowground biomass relationship, however management did (r^2=0.43, F=3.68, p=0.05. Figure 91).

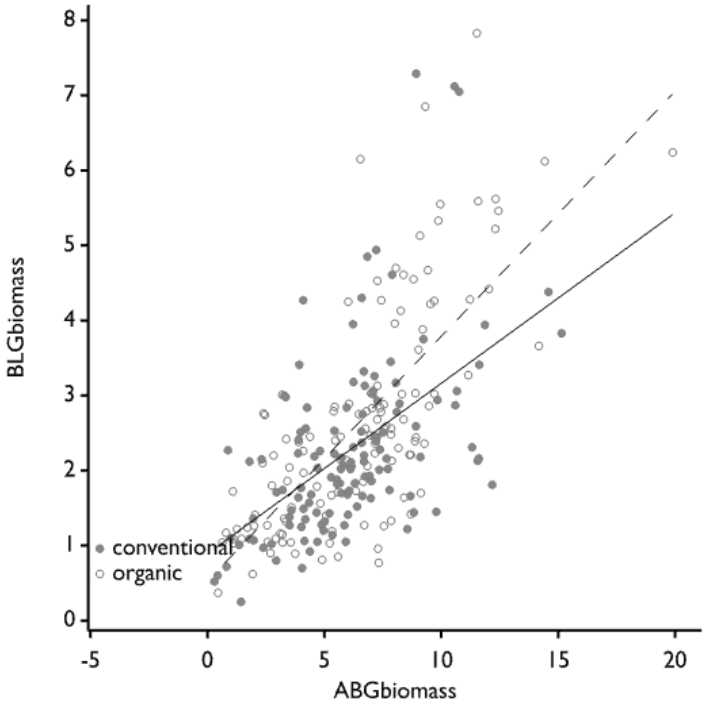


Figure 91 Scatter plot of belowground biomass as a function of aboveground biomass for both soil treatments

5.3.4 **Discrimin**ation between organic and conventional soils whilst including pest experiment variables

In order to test if the discrimination was improved between management by using pest experiment variables, a second set of discriminant analyses were produced using all soil parameters as well as mortality at 10 days from the soil baiting experiment and all variables from the inoculation experiment. Results are shown in Figure 92 and Figure 93 below.

Optimal variables	Counts	management		
WormAverage		conventional	organic	Total
Mortality_10D	allocated			
Mineral_NO3_N	conventional	18	.	18
BiomassC	organic	-	17	17
%_DM	Total	18	17	35
ABGbiomass	**No misclassification**			
BLGbiomass				
AWCDt5				
FLN_count				
pH				

Using bootstrapping with 632 rule to calculate errors **Error: 5.66%**		
Percentage of each group allocated to groups		
	True group	
Decision	conventional	organic
conventional	95.11	6.65
organic	4.89	93.35

Figure 92 Discriminant analysis (forward selection) using 10 variables for all soils sampled except Shef2 (no mineralizable NH4 N value), including soil and pest experiment variables, showing reclassification and errors

Optimal variables	Counts	management		
WormAverage		conventional	organic	Total
mortality_10D	allocated			
Mineral_NO3_N	conventional	16	1	17
damage	organic	2	16	18
ABGbiomass	Total	18	17	35
	Ab1 missclassified as organic			
	Dev4 missclassified as conventional			
	Linc1 missclassified as organic			

Using bootstrapping with 632 rule to calculate errors **Error: 12.47%**		
Percentage of each group allocated to groups		
	True group	
Decision	conventional	organic
conventional	88.54	13.6
organic	11.46	86.4

Figure 93 Discriminant analysis (forward selection) using 5 variables for all soil sampled except Shef2 (no mineralizable NH4 N value), including soil and pest experiment variables, showing reclassification and errors

Reclassification error using 10 variables was drastically reduced from 16% to 5% by including mortality at 10 days as well as both plant biomasses and no soil sample was misclassified. Reclassification error using five variables improved from 17% to 12% by adding mortality at 10D again, as well as aboveground biomass and damage (Table 55).

Table 55 Optimal variables for DAs with soil variables only compared to soil and pest variables

Optimal variables selected when pest experiment variables are included		Compared to optimal variables selected for soil parameters only	
10 variables 5.66% error	5 variables 12.47% error	10 variables 16.53% error	5 variables 17.57% error
WormAverage	WormAverage	WormAverage	WormAverage
Mortality_10D	**Mortality_10D**	Mineral_NO3_N	Mineral_NO3_N
Mineral_NO3_N	Mineral_NO3_N	BiomassC	
BiomassC	**Damage**	BiomassN	
%_DM	**ABGbiomass**	AWCDt5	
ABGbiomass		DOC	
BLGbiomass		pH	
AWCDt5		%_DM	
FLN_count		%_LOI	
pH		FLN_count	

5.4 Discussion

5.4.1 Organic management had some positive impacts on soil and plants, reduced the model pest survival but did not clearly enhance root pest suppression.

The organic managements represented in our soil survey impacted soil biological activity positively and increased soil organic matter, which was the only measured abiotic parameter significantly impacted. Perhaps surprisingly, no significant difference was found between managements for mineral NO_3 N. Even when covering such a breadth of practices, the simplistic organic VS conventional dichotomy was still relevant when considering the soils included in this survey, as shown by the discriminant analyses. The model pest died quicker in organic than conventional soils, potentially thanks to increased entomopathogenic nematode presence. In controlled conditions, plants grown in sampled organic soils developed a larger root system without any significant reduction of top biomass compared to the plants grown in conventional soil. Organic management reduced the number of pupae extracted from the root systems but only when larvae could complete their lifecycle. It failed however to dampen herbivory damage. As soil sampling was not replicated, our analyses can only provide a snapshot of pest-soil interaction and no reliable link could be found between soil parameters and pest suppression without repeated sampling. The inclusion of model pest mortality and plant biomasses did however improve the discrimination between systems, showing the added benefits of including plant and pest variables whilst considering the soil as a supporting system when comparing farming practices. While organic managements surveyed were beneficial for the crop's root system, the higher biological activity linked to those managements did not clearly lead to a consistently stronger pest reduction.

5.4.2 Management impacts on soil parameters

As our soil survey only provides a snapshot of the state of those soils at one single point in time, soil analysis results need to be considered with caution. Parameters were measured mainly to inform on management impacts on soil in the context of pest suppression and we cannot attempt to detail wider soil functions and processes. Nevertheless, organic management was clearly shown to have a positive impact on soil biological activity across taxa, as well as soil organic matter. Previous studies were not

always able to show clear differences (see Stolze et al., 2000) especially concerning organic matter (Armstrong Brown et al., 2000; Hathaway-Jenkins et al., 2011). Interestingly, Hathaway-Jenkins *et al.* (2011) justified the lack of difference in soil organic matter by the low application rate of farmyard manure on their sampled farms, compared to the 65 t/ha suggested to make a difference (Bhogal et al., 2009). Our sampled organic farms rate however varied from 10 to 37.5 t/ha, which was close to the 42 t/ha limit imposed by the UK government (DEFRA, 2018) and was enough to lead to an average increase of 15% in SOM compared to the conventional neighbouring fields. As SOM plays a major role in sustaining the rest of the soil food web (Scheu, 2002), it might not be surprising to also find that organic management led to an overall higher soil biological activity, both for earthworms and microorganisms. The positive impact on earthworms abundance is in line with previous research (Domínguez et al., 2016; Scullion et al., 2002) however we did not consider their total biomass, which could have led to further contrasting results (Scullion et al., 2002). No significant increase in biomass C and N was detected in organic soils though, contrary to expectation (Fliessbach et al., 2000; Hartmann et al., 2015, 2006). Organic amendments have been linked to an increase in pathogenic nematodes (Thoden et al., 2011) which was not shown here, as organic soils contained fewer free-living pathogenic nematodes. More broadly, the differences found here highlight the fundamental difference in fertility management between systems, as organic systems need to rely greatly on biological processes to release nutrients in a form usable for plants, thus requiring greater biological activity and diversity to function (Stockdale et al., 2009, 2002). This increased activity could potentially lead to enhanced plant defences and pest suppression.

5.4.3 Even with varied practices, the dichotomy organic VS conventional is here still relevant.

This project regularly contrasts organic and conventional farming practices. Sampling 36 farms however clearly highlighted the great diversity of practices used under each system (Table 45). Organic practices are usually defined and regulated through certifications such as Soil Association Standards (The Soil Association, 2019a), EU regulation (European Parliament, 2007) or internationally through IFOAM (IFOAM, 2014). Conventional agriculture, on the other hand, seems devoid of prescriptive technical content (Le Campion et al., 2020) and tends to be defined more in opposition to other labelled systems. Limitations in comparing those systems have been identified in varied contexts such as economics (Lobley et al., 2009), plant breeding (Le Campion et al., 2020),

biodiversity abundance (Bengtsson et al., 2005; Fuller et al., 2005) and natural enemies (Puech et al., 2014). Even if regulated, organic systems cannot be considered homogeneous (Stockdale et al., 2009) and Petit and Aubry (2016) clearly showed the heterogeneity of practices across production systems and over time. Using discriminant analysis on measured soil parameters allowed us, within the limit of our farms sampled, to test the validity of this dichotomy to qualify soil as well as identify which variables optimally discriminate the systems. Overall, organic and conventional samples could be discriminated, with fewer than 20% errors.

Top discriminating variables between organic and conventional soils were mainly biotic parameters, with SOM being last, somewhat contrasting with another paired study in England which used abiotic parameters only (Armstrong Brown et al., 2000). The second most powerful discriminating variable was mineral NO_3 N, which was not correlated to any other soil parameters. Even though there was no significant difference in mineral NO_3 N content between systems, this variable was one of two optimal discriminating variables selected by the five variables DA, along with earthworms count, with only a 1% error increase with the 10 variable DA. This tends to show the importance of complementary uni and multivariate approach when considering systems such as soils, as univariate analysis might only offer a limited perspective on differences between treatments.

Using ten discriminating variables, samples Dev1 and Shef1 were both misclassified as organic. In terms of management, both were fairly atypical of other conventional practices included in this survey. Dev1 only used mesh and nets with practically no pesticides in the last 20 years and managed their fields extensively, including long rotations due to the local presence of clubroot. Shef1 site used only a limited amount of pesticides, pig slurry every autumn and only minimum tillage as the field slope was fairly steep. Some recent research efforts have focused on the use of typically organic practices in conventional systems, such as ley and manure (Albizua et al., 2015), diverse landscape and biotopes (Mander et al., 1999) and agri-environment schemes (Marja et al., 2014) to reveal some promising positive impacts. In the context of our soil survey, organic and conventional samples did differ, even when representing such a variety of practices, similar to studies carried in wheat systems (Le Campion et al., 2020; Puech et al., 2014). Perhaps similar to wheat systems, vegetable production practices might be clearly linked to either organic or conventional approach, enough so to impact the soil in different ways. In

parallel, misclassifications also highlight the existence of a gradient of practices in conventional farms, which could lead to the blurring of differences with the positive impact of minimum tillage, organically based fertilisation or reduced pesticide used.

5.4.4 Management had a stronger impact than physical pairing

Physical pairing within the landscape had a very limited influence compared to management on measured parameters as only four pairs out of 18 were cluster neighbours. Soil type will have a strong influence on starting soil conditions, especially texture (Hathaway-Jenkins et al., 2011) but subsequent management has been shown to clearly affect the soil in the context of paired studies, even when focussing on more physical parameters (Armstrong Brown et al., 2000). This strong signal of impact of management at local scale resonates with results from our experimental field sites, where soil parameters were shown to differ within a few meters at plot level between organic and conventional management (Orr et al., 2012, 2011; Reilly et al., 2013). Even with less optimal starting conditions, organic management can have a positive impact on the soil as habitat at sub-field scale.

5.4.5 Organic management reduced model pest survival more quickly than conventional management, potentially thanks to the presence of entomopathogenic nematodes.

No sterile control was included in the baiting with survey soils, whilst soil sampling was not replicated, thus pest experiment results can only represent a snapshot of the possible impact of management. With those limitations in mind, a few differences between organic and conventional management impact can be highlighted. Overall, organic management led to almost twice as many dead larvae at 10 days. However, similar to Kinsealy soils, the gap closed and the difference in mortality became only a trend at 19 days ($p=0.07$). When considering the paired approach used along with survival analysis, this higher organic mortality was present in ten pairs out of 18, compared to 4 pairs where conventional management led to higher mortality. Considering the conventional management in those pairs in more details, a few potential clues could help explain those differences, thanks to management impacts previously highlighted in entomopathogen research (Clifton et al., 2015; Klingen et al., 2006; Uzman et al., 2019). Ab1 benefited from the regular addition of farmyard manure at 25 t/ha rate (higher than some organic sites), pig slurry was applied every autumn in Shef1, in parallel with minimum tillage, whilst Linc1

site used mustard as green manure very regularly due to the local presence of potato cyst nematodes. No particular atypical management was identified for Corn3 however, which was even regularly double cropped with Brassica under an intense pesticide regime. Considering those paired differences and the local impact of management on soil parameters, it could be argued than adequate management could foster an enhanced microbial based pest suppression, perhaps more easily than with predators and parasitoids, whose increased abundance can only be achieved if the right local metapopulation is already present (Tscharntke et al., 2016).

5.4.6 Presence of entomopathogens and link to mortality rates

Even if presence/absence data is only of limited use, our analysis has shown that organic samples tended to contain nematodes more often ($p=0.06$), in line with more precise previous studies (Campos-Herrera et al., 2010, 2008; Williams et al., 2013). Sporulating fungi presence was once again very low, similar to Chapter 4, and no difference was found between management. As discussed previously in Chapter 4, the organic VS conventional dichotomy might not be relevant when surveying entomopathogens, with soil physical factors (Quesada-Moraga *et al.*, 2007) or tillage (Bing et al., 1993; Clifton et al., 2015; Hummel et al., 2002a) potentially being more relevant . The lack of species identification and reinfection test also restricts the information provided by those results even more. Nevertheless, two pieces of information could inform our system functioning. Firstly, when considering our survival analysis and the rate of survival of the model pest, it can be observed that some organic soils showed a very strong impact early on, where the majority of larvae died in three days (Annex 14, full t test and survival curves). Without species information and with the limited relevance of presence/absence data, no clear conclusion can be reached, however those samples all contained nematodes. In terms of speed of suppression, our correlation indicates that mortality at 10 days was correlated with both type of pathogens recorded but mortality at 19 days only tended to be correlated with fungi ($p=0.06$).This could point towards the quicker capacity of nematodes to suppress root pests compared to fungi, as nematodes have been shown to suppress fungi (Ansari et al., 2005). Even if limited by the baiting technique used and the incubation conditions, nematode and fungi presences were indeed here negatively correlated at -15%. Unfortunately, those results cannot be compared to previous soil baiting entomopathogen studies including both type of pathogens (Hummel et al., 2002; Chandler and Davidson, 2005; Tkaczuk et al., 2014) as those studies neither included mortality rate over time nor

the discussion of pathogen competition in *Galleria* hosts. Studies focussing on the interactions of entomopathogens also report either additive effects (Barbercheck et al., 1991), additive or antagonistic effect depending on species combinations (Shapiro-Ilan et al., 2004; Wu et al., 2014), or clear antagonism (Ansari et al., 2005), but none of those studies were based on natural soil habitat and its baiting but based on artificial inoculations.

5.4.7 Organic management led to improved plant growth without clearly impacting the pest negatively

Plants grown in organic soils developed a larger root system, without any negative impact on their aboveground biomass. In comparing organic and conventional systems, a yield gap tends to be expected between management but clearly depends on local growing conditions (Cunningham et al., 2013; De Ponti et al., 2012; Ponisio et al., 2015; Schrama et al., 2018; Seufert et al., 2012; Stanhill, 1990). Plants were grown in very artificial conditions and sampled destructively before maturity, so the lack of difference in aboveground biomass can only point towards a potential lack of yield gap. Concurrently, a larger root system could help the plant withstand more root herbivory without any reduction of yield potential. This link was not identified here, as inoculation impacted organic and conventional plants in the same way, both in terms of aboveground biomass weight and damage score. No clear sign of enhanced bottom up suppression in more active organic soil was shown here.

Even though grown in larger pots, root systems yielded very few pupae once again. Inoculation was successful and larvae did at least somewhat develop as herbivory damage was clearly present on the majority of inoculated plants, but larvae must not have been able to complete their life cycle. When analysing the whole inoculated dataset, no difference in pupal numbers was found between managements. When considering the non-zero dataset, in pots where at least some larvae did complete their lifecycle successfully, -63% pupae were extracted from organic soil compared with conventional soils. This contrasts with our experimental field soils results from Chapter 4 on a restricted dataset, as plants grown in organic soil from Kinsealy had more pupae, whereas Nafferton soils showed no difference. This result is of limited impact as only non-zero counts are considered, but could point towards a stronger pest suppression in organic soils. One part of the puzzle is still missing though as once again weighing individual pupae would have added information

on potential impact of management. As contrasting fertility managements can have an impact on herbivory (Altieri et al., 2003; Alyokhin et al., 2005; Eigenbrode et al., 1988; Meyer, 2000; Scriber, 1984), we would have expected a difference in damage score between management, however no significant difference in damage was identified between management regimes. In terms of pest-plant interactions, damage was correlated positively with aboveground biomass, belowground biomass, and also number of pupae. Against expectation, our data showed that a larger plant sustained more damage and allowed the development of more pupae.

5.4.8 Linking soil parameters to root pest suppression

As this soil survey was not replicated and pest experiments using those soils were not either, all variables measured or produced only represent those systems at one moment in time, and any link found between pest experiment variables and soil variables would have to have been considered with caution and pushed our data analysis too far. Pupal counts also potentially contained too many zeros to really be informative and be linked to soil parameters. The inclusion of some of the pest suppression experiment variables however greatly improved the discrimination between soils: the best organic VS conventional discrimination, with no misclassification and only 5% error, was with the addition of mortality at 10 days as well as both biomasses. This result once again highlights the importance of the perspective added by multivariate analysis, as well as considering the impact of management on the soil sustaining a wide variety of functions.

5.5 Conclusion

The range of organic managements surveyed had an overall positive impact on the soil, mainly through the increase of soil organic matter and higher soil biological activity. The organic soils also reduced the model pest survival significantly quicker than conventional soil, with some killing the model pest within a week, while our soil baiting analysis demonstrated the potential negative interactions between entomopathogens and the relevance of considering mortality over time. Our inoculation experiments failed once again to show a consistently enhanced suppression of our root pest by organically managed soil. From a soil-plant perspective, organic soils led to larger plant root system which could be precious in field situations, yet without any significant negative impact on the

aboveground biomass, pointing towards a potential lack of final yield gap between managements. Multivariate approaches allowed us to gain a better understanding of our sampled soils as systems and showed the relevance of still contrasting organic and conventional practices in our study, while the lack of entomopathogenic species identification and soil sampling replication truly limited the impact of our results. Even if only providing a snapshot of how plants and pests can interact with soils from contrasting practices, we were still able to show the deep impact that management can have locally on similar soils, also impacting plant growth and the pest suppression potential, with organic management potentially leading to wider benefits for the soil as a complex habitat sustaining a wide arrays of ecosystem services.

Chapter 6 General discussion

6.1 Integrating soil in conservation biological control of root pests

Global food systems are evolving in order to sustainably provide for a growing population (Garibaldi et al., 2019; Gunton et al., 2016; Pretty et al., 2018). Guiding this transition, the concept of sustainable intensification is now included in high level policy frameworks such as the UN's Sustainable Development Goals for 2030 (UN, 2015) and the recent European Union Farm to Fork strategy (European Commission, 2020). Sustainable intensification relies on the enhancement of synergistic ecosystem services to foster multifunctional and resilient agroecosystems (Bretagnolle et al., 2018; Dainese et al., 2019; Lefcheck et al., 2015; Tittonell, 2019). Soil health and integrated pest management have both been identified as key elements to reach those goals (Tilman *et al.*, 2002; Roger-Estrade *et al.*, 2010; FAO, 2015c; Crain, 2016; Robinson *et al.*, 2017; Zhang *et al.*, 2018a; European Union, 2020), yet they are unfortunately rarely considered together and only a limited amount of research has investigated links between soil health and pest management (Altieri et al., 2005b; Bender et al., 2016; Stavi et al., 2016; Stelinski et al., 2019). Synergy between ecosystem services within remodelled agroecosystems could be harder to reach if the soil, as such a large component of the landscape puzzle providing a wide range of essential services (Haygarth et al., 2009; Huguenin et al., 2006), is not also included in integrated pest management strategies. At the heart of integrated pest management, conservation biological control should benefit from the inclusion of the soil in its framework, as a habitat but also as a reservoir of pest natural enemies (Klingen et al., 2006), especially when investigating the regulation of root pests, as shown in Figure 94. This thesis attempts to demonstrate how including the soil in the investigation of farming practices impacts on conservation biological control can provide additional clues on enhancing root pest suppression.

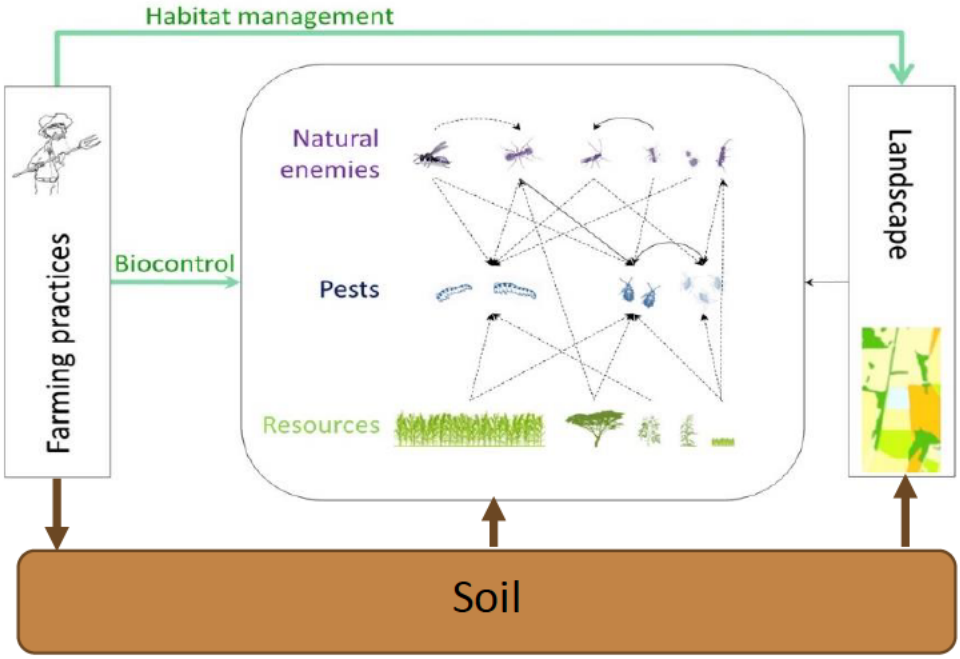


Figure 94 Conservation biological control framework including soil - adapted from Brévault & Clouvel, 2019

6.2 Farming practices impact on soil

Considering more than soil management practices

Without always being labelled as such, a wide range of farming practices such as crop protection or crop rotation do indeed impact the soil and its community of organisms as shown in Chapters 2, 3 and 5 of this thesis. As our main hypothesis was linked to organic management's potential to enhance soil biodiversity and natural enemies' presence, demonstrating organic and conventional management impacts on soils was the logical first step before considering pests and natural enemies. Main elements from soil analyses across chapters are summarised in Figure 95. Whilst not carried out as thoroughly as in our survey chapter, our own soil analysis and published literature were able to demonstrate the impact of managements on the soils of our experimental rotations. Organic management did positively impact soil microbial activity in both sites but had unexpectedly limited impact on soil carbon and nitrogen and did not increase either as expected (van Eekeren et al., 2009). Similarly, the wider range of organic practices considered more in depth in Chapter 5 did also positively impact soil microbial activity, as well as earthworm activity, but did not lead to expected higher carbon pools, apart from soil organic matter, or lower

mineral nitrogen. The main impacts of organic managements included in this study was then centred around increased soil biological activity, with limited impacts on abiotic parameters, thus highlighting the need to consider more than dedicated soil management practices' impacts such as tillage or fertilisation when including the soil in agroecological studies. This enhanced activity in organic soils does not inform however on soil biodiversity or wider food web dynamics.

Soil biological activity and pest suppression

Enhanced microbial activity in organic soils was somewhat expected as organically fertilised soils have to be more active in order to release nutrients, unlike minerally fertilised soils (Stockdale et al., 2009). In Chapter 5, organic managements led to larger root systems, showing a positive impact on the wider plant-soil system. However, no link was identified in our study between this enhanced activity and the pest suppression potential of the soil, not even through better plant health. In the wider food web context, this enhanced microbial activity might however have been part of the elements leading to higher root system and epigeal natural enemies' activity in our experimental rotations, as this activity could have been the base for a richer food web. This however cannot be confirmed and better assessment of the soil food web in those fields would have provided precious insight (see Birkhofer et al., 2008; de Vries et al., 2013) as conservation biological control needs to be considered across trophic levels (Tscharntke et al., 2007; Brévault and Clouvel, 2019). Indeed, higher soil microbial activity on its own might not be beneficial in our pest suppression context as soil community composition, rather than activity, has been identified as a key factor in supporting varied ecosystem services (Bender et al., 2016; Wagg et al., 2014). Including the evaluation of earthworms and pathogenic nematodes presence was not adequate to further qualify soil biodiversity and more planning and effort should have been dedicated to the assessment of diversity indexes across selected functional groups, similar to Tsiafouli et al. (2015) who considered earthworms, Collembola, oribatid mites and nematodes using richness and Shannon indexes. Even though soil biodiversity is clearly worth enhancing and protecting (Altieri et al., 2012; Amundson et al., 2015; Bulgarelli et al., 2012; European Union, 2020; FAO, 2015b; Mulder et al., 2011; Nielsen et al., 2011; Orgiazzi et al., 2016; Wall et al., 2015), enhancing it blindly without considering community composition might not enhance biological functioning due to functional redundancy among species (Nielsen et al., 2011) and care should be taken in order to optimize those communities to sustain ecosystem multifunctionality (Bender et al., 2016).

Lemanceau et al. (2014) call for the integration of awareness of farming practices impact on microbial abundance, diversity and activity into farming systems redesign, while using ecological engineering in order to orient and favour beneficial microbial communities, also including inoculation as a possible tool. While this level of control might not be possible yet, we fully agree that this awareness would be beneficial to aim for ecosystem multifunctionality, including in conservation biological control.

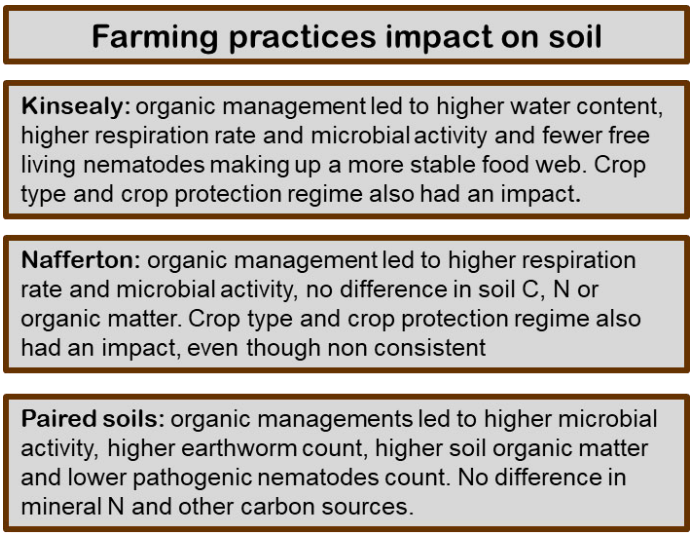


Figure 95 Summary of farming practices impact on soil, for Kinsealy, Nafferton and commercial field soils

6.3 Organic managements can enhance conservation biological control of root pests: main lessons learned

For clarity, a graphical summary of the main results of the thesis are included below (Figure 96 and Figure 97).

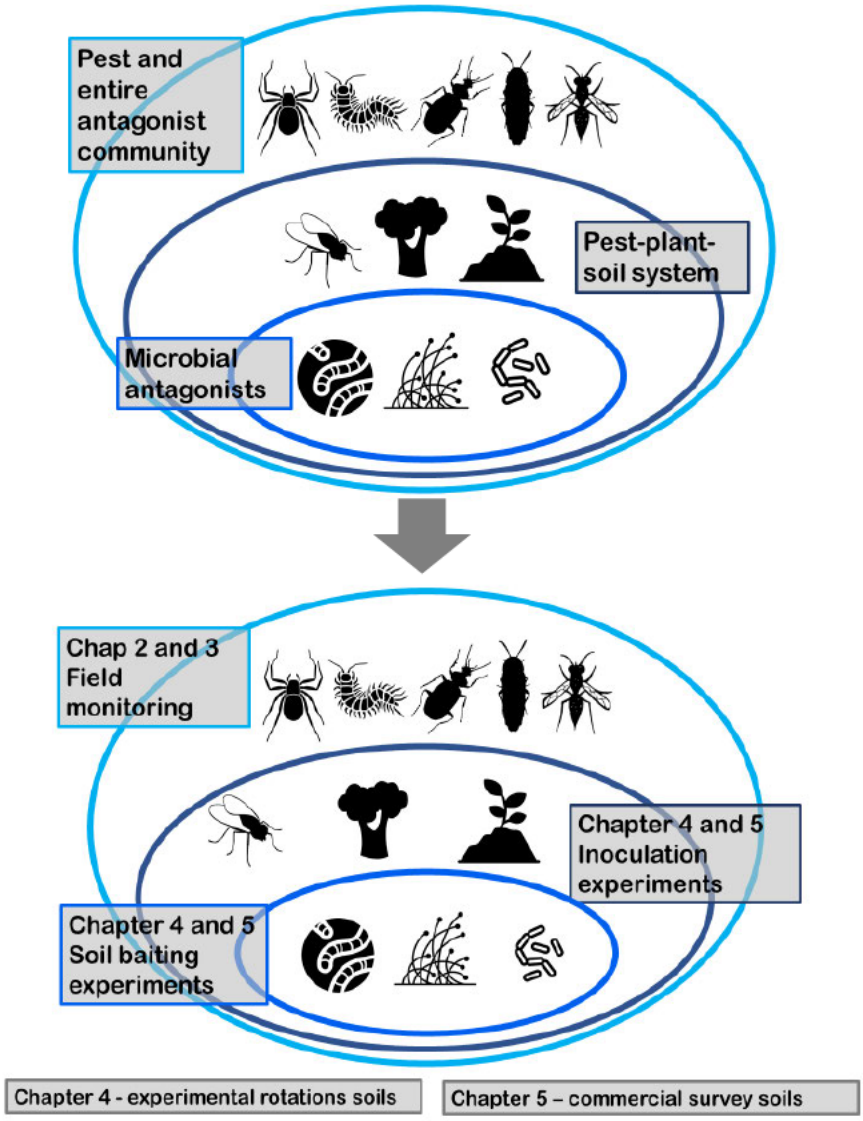


Figure 96 Overall thesis structure

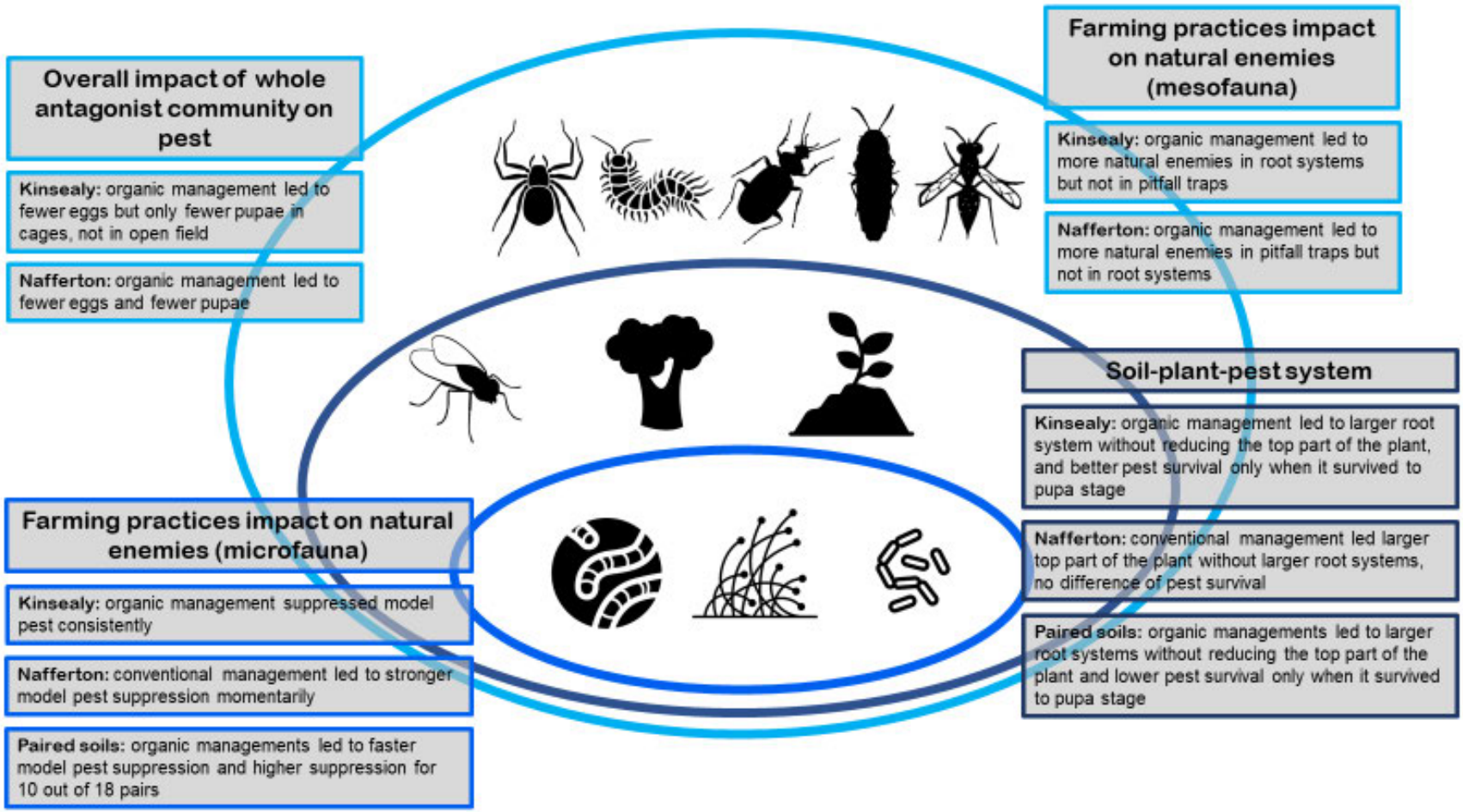


Figure 97 Graphical summary of main thesis results

Can we learn from plot level ecology?

As previously highlighted in Chapter 2 and 3, experimental rotations' plot scale is clearly not an adequate scale to assess pest and natural enemies dynamics (Furlong et al., 2010). As samples are not independent, we can only show preferences of those organisms belonging to the same metapopulation and only very short range impacts of farming practices on their activities. The enhanced pest suppression and natural enemies' presence in organic plots in Kinsealy and Nafferton will most likely not transfer to commercial, large scale, mono-cropped fields and generalising those positive impacts to larger, more adequate scales would be unwise. However, two main points that emerged from our plot scale results are still relevant above plot scale and could inform future field-scale conservation biological control work.

Conservation biological control manipulations and practices can lead to increased natural enemy presence but sometimes fail to improve pest suppression (Dicks et al., 2016; Nilsson et al., 2016; Tscharntke et al., 2016a) highlighting, amongst other issues, the need for better spill over and movement of natural enemies within the crop itself and in the root system itself. In Chapter 2 in Kinsealy, the predators present in the broccoli root systems were positively impacted by both semi-natural habitat proximity and organic management, pointing towards the potential of utilizing both to draw more predators into infested root systems. If organic managements in Kinsealy and Nafferton led to significant differences in root pest and natural enemies presence at such a short range, it could be argued that adequately managed soil could promote this spill over and act as a bridge between natural enemies reservoirs such as semi natural habitat (Holland et al., 2016; Bartual et al., 2019; Mchugh et al., 2020), the crop that needs protected and its root system where the pest needs to be suppressed.

The second point is linked to the complementary sampling of epigeal community and root system community. In Kinsealy, organic management led to higher root system predator presence without leading to higher epigeal predator activity density at plot level, whereas opposite results were found in Nafferton, highlighting the different local dynamics and repartition of overall local antagonists' community, which can provide precious information on antagonist community management. Including a cage experiment in Chapter 2 also contributed to understanding the lack of pupae suppression in open field even though more predators were present in the root systems, by comparing it to the

enhanced pupae suppression found within the cages. As cages removed larger intraguild predators such as *P.melanarius*, they potentially led to the improvement of the complementarity of the predator community, necessary for effective pest regulation (Perović et al., 2017; Snyder, 2019) This complementary sampling also highlighted the clear need to actually assess the enemies co-occurring with the pest and not just limiting the sampling to the assessment of the epigeal activity density with pitfall traps, as those did not adequately capture the abundance and community makeup of the enemies co-occurring with the various life stages of pest sampled. Along with selecting the adequate scale to study pest regulation dynamics, it is also necessary to "observe differently", using complementary approaches to encompass the wider systems involved in this pest suppression (Brévault et al., 2019). Even only at plot scale, outside commercial settings, highlighting the ubiquitous presence of known natural enemies of *Delia radicum* within pest samples, as well as showing the ever-present resource-consumer link, contributed to making this pest suppression potential more visible. This visibility could then contribute to raising the profile of natural regulation and its inclusion into individual and collective decisions regarding pest control (Opdam et al., 2016) while contributing to providing a stronger scientific basis for managing functional biodiversity (Letourneau and Bothwell, 2008) within the framework of conservation biological control.

Including microfauna in conservation biological control

Chapter 4 may not have revealed consistent differences between microfauna pest suppression in organic and conventional soils, however, it clearly showed the impact of field soil on the survival of our model pest compared to their sterile counterparts. The similar soil baiting carried out in Chapter 5 revealed the potential of some field soils to suppress the model pest totally within a week without the presence of mesofauna while the inoculation experiments pointed towards the positive impacts of surveyed organic soils on the root systems without penalising the top part of the plant. Those results show that conservation biological control strategies for root pests would benefit from the inclusion of microfauna such as entomopathogenic nematodes, fungi or potentially predatory mites, while also considering larger predators and parasitoids. To our knowledge, very few studies have focussed on this part of the antagonist community, such as entomopathogenic nematode management in citrus groves in Florida (Stuart et al., 2008) and those also advocate the inclusion of the entire guild of enemies when developing conservation biological control strategies (Pell et al., 2010).

Stacking up soil ecosystem services

While organic managements in Chapters 2 and 3 had only a limited impact on abiotic parameters, they all tended to lead to higher biological activity, across different taxa and reduced pest survival while enhancing natural enemy presence at plot level. The pesticides used in those rotations were also shown to have a detrimental impact on the crop itself (bleached broccolis in Chapter 2) and *D. radicum* natural enemies (chlorpyrifos negative impact in Chapter 3). In Chapter 5, organic managements led to larger root systems, faster model pest suppression, higher earthworms count as well as lower pathogenic nematodes counts and reduced *D. radicum* incidence when it survived to pupate. Even if this study has struggled to show a clear link between management impacts on the soil and enhanced pest suppression, those results need to be considered within the wider soil health and functioning framework, as part of the agroecosystem puzzle. If sustainable intensification requires harnessing synergistic ecosystem services to sustainably improve agroecosystem productivity (Bretagnolle et al., 2018; Dainese et al., 2019; FAO, 2016; Firbank et al., 2013; Lefcheck et al., 2015; Tittonell, 2014), the implementation of managements leading to healthier, more diverse soils will lead to more than just improved productivity, also participating to the necessary restoration of agroecosystems integrity (Alyokhin et al., 2019) and potentially improving plant, animal and human health (Wall et al., 2015). As research is starting to look beyond the simplistic opposition of organic and conventional systems, the inclusion of organic practices in conventional systems (Albizua et al., 2015; Pimentel et al., 2005), conservation agriculture (Chabert et al., 2017; Pretty et al., 2018) or ecological/low input agriculture (Mander et al., 1999) all appear to offer opportunities for improving soil health and nurturing multifunctional agroecosystems.

Variability and reliability as barriers for adoption

Similar to other studies investigating the regulation of *Delia* (Björkman et al., 2010; Meyling et al., 2013; Nilsson et al., 2016), our study also revealed intra and inter-year variability of pest and natural enemies presence (Chapters 2 and 3), as well as variability of the pest suppression by the soil microfauna (Chapters 4). The within-site variability and different functioning of our rotations highlighted in Chapter 3 constitute a large hurdle for the adoption of conservation biological control strategies by farmers. Research has so far struggled to prove the reliability and efficacy of habitat management strategies for pest management (Dicks et al., 2016) and our study also highlight some potential breakdown of natural pest suppression, for example when higher predator numbers in root systems did

not lead to improved pest suppression in open field in Chapter 2, potentially because of intraguild predation. The lack of reliability of conservation biological control is still its greatest limitation, as highlighted by Begg et al. (2017), as well as its requirement for thorough knowledge of the community ecology dynamics at play. This can lead risk-adverse farmers to only have limited confidence in this strategy (Zhang et al., 2018) even when implemented within a wider resilient IPM approach, as the necessary collaboration at higher scale for effective open field IPM still poses risks (Tracy, 2015).

Learning from surveyed farmers: other barriers to adoption

Even though our resources were limited, we clearly missed a precious opportunity to quantitatively survey the farmers and agronomists who agreed to be part of our soil survey in Chap 5, which could have helped to get a better insight in commercial practices, decision making and knowledge gaps. Despite this missed opportunity, we believe a few points highlighted during our casual conversations offer very valuable insights of further barriers to adoption for conservation biological control and sustainable soil management. The first point to report, made by the majority of organic and conventional farmers alike, was the issue of limited availability of farmyard manure, with some farmers begrudgingly resorting to the use of municipal green waste compost or green manure instead. Even if self-selected with a clear interest in soil health, most of the conventional farmers surveyed also clearly wanted to integrate manure in their soil management and were keen to use more than they could only sporadically source. The second point is linked to land tenure. Even non-tenant farmers seemed to rely heavily on rented land for their brassica crop rotation and could not effectively plan ahead, as collaboration between neighbours seemed very limited. When discussing improving soil health and pest management, only farmers managing their own fields seemed confident to be able to put a 2-5 year plan in place, which would be the time scale required to see an effective change in soils (European Union, 2020; Jabbour et al., 2009; Lundgren et al., 2006). The cost-benefit aspect of natural regulation was obviously an overbearing issue, as the combination of limited economic visibility for farmers and limited economic valuation available (Cullen et al., 2008; Naranjo et al. , 2015; Shields et al., 2019; van der Werf et al., 2020) led farmers to question the soundness of this strategy choice. Farmers also highlighted their lack of knowledge about natural enemies and which were present on their farms. Even when keen to learn, they struggled to find the time and support to make progress. The majority of barriers

highlighted by this small, self-selected sample of farmers and agronomists are also reported by published research as discussed in the Future Research section (6.6).

6.4 Limitations of research carried out

While conceptualisation and implementation limitations were highlighted across the different chapters, we would like to highlight broader issues impacting the potential impact of this thesis.

While we believe our community approach is valid and novel, it unfortunately led to the main weaknesses of this study. By including the entire antagonist community of *Delia radicum,* we were not able to produce species level data and adequate replication of natural enemy sampling. Without species level identification, local ecosystem functioning has stayed blurry and a few of our results unexplained. More importantly, we were not able to produce diversity measures and assess both soil and natural enemies' diversity, as only abundance data was produced along with very limiting natural enemies' functional groups. If fewer taxa were included, we could have adopted a functional approach (Letourneau et al., 2008) to determine antagonist community traits necessary for effective root pest control, including trait complementarity (Snyder, 2019). This approach has been shown to be effective for deciphering belowground food web and ecological dynamics through projects like BETSI, a French database for soil invertebrate biological and ecological traits (portail.betsi.cnrs.fr). In terms of replication, pitfall trap sampling should have been carried out for both years monitored, and not limited to the egg sampling period, as we believe this would have increased our chance to show an actual pest suppression link between enhanced natural enemy presence and the different *D. radicum* life stages.

This thesis attempted to make a case for the inclusion of the soil in conservation biological control studies, however this case might have been stronger if more effort were spent to assess farming management impacts on the soil. Even though Chapter 5 included a thorough soil analysis, Chapter 2 and 3 did not, and we missed the opportunity for replicated soil sampling during both monitored years, which could have produced a sounder base for the further pest and plant experimentations of Chapter 4. Biotic parameters determined were limited and a more thorough food web assessment could have helped investigate trophic dynamics and potentially linked the farming practices impacts on the soil to the enhanced pest regulation. Though gut content analysis of

predators sampled (Birkhofer et al., 2017; Harwood et al., 2005; Lundgren et al., 2011) would have been the best approach to reveal direct pest suppression links, a wider food web approach similar to Tsiafouli et al. (2015), who assessed the diversity of earthworms, Collembolans, mites and nematodes to reveal the negative impact of intensive agriculture on soil biodiversity, would have truly highlighted the farming management impacts on soil biodiversity and functioning. As a large amount of effort and resources was dedicated to the surveying of commercial farms' soils, a more detailed soil food web analysis, as well as entomopathogenic species, could really have pushed this soil set further. Too large in scope to be carried out effectively, this study would have greatly benefited from being either split in two joint studies, with one study focussing on pest suppression through mesofauna and the second focussing on microfauna, or from wider departmental collaboration in order to investigate complementary aspects of the soils and sites sampled, including elements of landscape or chemical ecology.

Issues linked to plot scale and unavoidable field experimentation design limitations have been highlighted throughout this thesis. We believe that by moving away from experimental rotations and focussing on commercial farm sampling instead, we would have gained greater insight in realistic community ecology involved in root pest suppression, in actual farming landscape instead of the very artificial strip split plots. As comparing organic to conventional systems without recognising the diversity of practices within each category is not always the most relevant framework (Chabert et al., 2020; Puech et al., 2014), this study would have benefited from investigating more thoroughly the wider set of farming practices included in Chapter 5, whilst also learning from being in contact with farmers and agronomists. Experimental rotations are a safer playground for research students as management protocols are already in place and closely followed by trained research farm staff, compared to commercial farms which have very different priorities. However, as commercial farms have to keep pristine records of field level management, detailed field history and in-depth agronomic details can be obtained, which would provide a solid basis for studying farming management impacts on soil, pest and natural enemies. This would have provided this study the adequate scale, as well as realistic field landscape conditions, thus improving its potential impact.

The last issues we would like to highlight are linked to the methods used. In addition to the issues around field experiment design, interspersion of treatment along a strip and

the pervasive pseudoreplication without any independent samples (Hurlbert, 1984) in experimental fields divided in plots, the over reliance on p values clearly limits this study. Non-independent sampling, which impacts estimates of effect sizes, could have been taken into account by pushing our statistical analysis further with sensitivity analyses, which help provide greater confidence in results as well as highlight important limitations of empirical work including the impact of study design on overall effects (Noble et al., 2017). The exclusive use of p values however cannot adequately reflect complex community ecology functioning as it tends to offer a black and white answer, but can still be relevant in ecology in some context (Murtaugh, 2014). Currently beyond our actual skills, Bayesian inference methods, which uses the information available before a study to build a quantitative model or hypothesis (Ellison, 2004), would have been more adapted to this work, as it uses a probabilistic approach a lot more suited to ecology, as well as dynamic decision making for agroecosystems management (see Brittan and Bandyopadhyay, 2019). We would hope that training in Bayesian statistics becomes available enough for ecologists of all levels to become familiar with this promising alternative approach.

6.5 Novelty of research carried out

As a PhD project, this study is an original piece of research, even if limited in scope and depth. We believe that by trying a different approach and methods, it contributes to pushing the envelope of conservation biological control of root pests in four ways highlighted below.

Community approach

This study has attempted to consider the impact of the entire antagonist community involved in the regulation of *Delia radicum*, including predators and parasitoids commonly studied while also including microfauna antagonists. By monitoring pest and mesofauna natural enemies in the field then using the root system soils to assess the suppression potential of the microfauna alone, we tried to break down and identify which part of the community had a significant impact on *Delia radicum* suppression, which has not been attempted before to our knowledge. Even if pest suppression links were not obvious, we at least recognised the possible impacts of those different parts of the antagonist community and their possible interactions, which has not been included in other *Delia* conservation biological control studies.

Complementary sampling

To our knowledge, this study is the first to investigate co-occurring natural enemies and pest, across the different pest life stages present in the soil and not just on the surface. By using complementary sampling and not only pitfall traps, this study was able to highlight the importance of some groups of predators, especially the ever-present medium size Staphylinid, despite the use of limiting functional groups, no species level data and limited replication. In terms of natural enemies, we believe that linking epigeal activity and community with root system activity and community is a worthwhile pursuit to improve our understanding of local community ecology and the lack of enhanced pest suppression despite increased numbers of natural enemies. Using a multivariate approach also helped consider the systemic nature of our sampling strategy and potentially highlight new ways of investigating root pests in future conservation biological control work.

Using soil baiting to assess soil suppression potential

Soil baiting with model pest is commonly used to identify entomopathogens in field soils, focussing on either nematodes or fungi. This study used this method in a novel way to assess the soil suppression potential, including all co-occurring microfauna antagonists present in field soils. Perhaps not as relevant as expected without species identification as well as the biased selectivity linked to the use of a lepidopteran bait, our community approach here was however once again novel. Additionally, the use of survival analysis, as a complement to end point mortality analysis, showed the additional information that can be gathered in this type of experiment and could add depth to future soil baiting experiment where speed of suppression matters.

Considering the plant-soil-pest system

While inoculation experiments are not new, using them to assess the impacts of farming practices on the soil, the host plant as well as the pest was a novel way of investigating this system under controlled conditions. Even though we were not able to explain the high mortality of the pest in the inoculated root systems, those experiments and our multivariate approach offered a novel way of investigating farming practice impact on plant health and growth, while impacted by herbivory, while linking those variables to soil parameters.

Focussing on the soil

The main novel element provided by this study is its focus on the soil. Instead of focussing on the commonly studied flower strips, semi natural habitat or any other aboveground habitat manipulation, this study looked belowground and attempted to link farming practice impacts on the soil to the suppression of a root pest. As previously mentioned, conservation biological control studies rarely consider the soil as part of the habitats that could be manipulated and included in management strategies. To our knowledge, this study is the first to attempt linking soil parameters to root pest suppression, in a wide range of field soils. Even with issues of scale and limited depth, this study has hopefully shown that including the soil in the investigation of the conservation biological control of a root pest is a valid approach that, if carried out more thoroughly, could provide precious information on agroecosystem functioning.

6.6 Looking forward: future research in conservation biological control

Sustainable intensification in the UK outside Europe: policy framework

After exiting Europe, the UK is currently in a period of transition, where key bills are being drafted in order to replace European law. Regarding agriculture, the "repatriation of competences" gives rise to strong differences in attitudes to agricultural support and policy implementation across the UK (Keating, 2019). Key laws are currently being discussed by parliaments, including the Agriculture Bill 2019-2021 and the Environment Bill 2019-2021 in England, and the Agriculture (Retained EU Law and Data) (Scotland) Bill. Concurrently, the European Parliament recently published its new Farm to Fork Strategy, as part of its new Green Deal framework policy (European Commission, 2020). Developed in order to implement the United Nations' Sustainable Development Goals (UN, 2015) and the Paris Agreements (UNFCCC, 2016), the EU Farm to Fork policy clearly highlights agroecology as a backbone for the transition to sustainable food system, similar to what the French government implemented in 2015 as part of its national "agroecological transition"[5] .This EU policy also sets up targets such as 25% of EU agricultural land under organic management by 2030 as well as a reduction of overall use and risk of chemical pesticides by 50% by 2030, while aiming to update its IPM directive (European Commission, 2020) and draft stronger soil legislation this time with binding targets (European Environment Agency,

[5] agriculture.gouv.fr/agriculture-et-foret/projet-agro-ecologique

2019). Current laws discussed in Westminster contrast starkly with this European political direction, as the Agriculture Bill 2019-2021 so far only mentioned agroecology once in a very diminutive manner[6] (UK Government, 2020). The two amendments that could have made a larger place for agroecology in this law have not been voted on yet due to the current COVID crisis (amendments 18 and 19[7]). The Environment Bill 2019-2021 is currently under public review in England until the end of July 2020 and includes the Environmental Land Management (ELM) scheme, to be implemented by the end of 2024 as a Common Agriculture Policy replacement (DEFRA, 2020b). This new agri-environmental scheme (AES) includes three tiers, the first of which mentioning habitat manipulation for pest management amongst other measures, the second aiming at encouraging and rewarding collaboration between farmers and/or land managers to ensure successful delivery of outcomes, while the third tier is reserved for landscape scale land-use change projects, afforestation and peatland restoration (DEFRA, 2020b). Nothing equivalent in Scotland was found at the time of writing. Those laws are all currently being either debated in parliament or under public review, and their content may evolve. Policy suggestions relevant for the UK sustainable intensification have been produced by diverse groups such as Sustain[8], the Soil association (Green Brexit, Soil Association, 2019), and international collaborations such as the International Panel of Experts on Sustainable Food Systems (IPES-Food, 2015), International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD, 2009) or Garibaldi *et al.* (2019). As the latter really resonated with us, their policy targets are presented below in Table 56.

Table 56 Science-based policy targets for ecological intensification, from Garibaldi et al., 2019

1- Enhance above- and below-ground species diversity.
2- Reduce synthetic inputs
3- Enhance soil health
4- Maintain or restore natural and semi-natural areas
5- Protect and efficiently use water resources
6- Enhance habitat diversity
7- Integrate practices into a landscape design
8- Evaluate agricultural productivity and ecosystem services over the long term
9- Consider multiple benefits
10- Facilitate participatory action and farmer training

[6] ""better understanding of the environment" includes better understanding of agroecology"
[7] services.parliament.uk/Bills/2019-21/agriculture.html
[8] www.sustainweb.org/foodandfarmingpolicy/agriculture_bill/

The current COVID crisis clearly highlighted the weaknesses of the UK food systems due to insufficient capacity in domestic food production, just-in-time supply chains and Brexit-related labour market challenges (Garnett et al., 2020). This pandemic also led to consumers and food producer relationships to evolved very quickly, with consumers prioritizing local food supply chains (Darnhofer, 2020; Hobbs, 2020) and community-supported agriculture schemes (Gemmill-Herren, 2020), which may have lasting effects on consumers expectations of the food production system. Researchers have been quick to make the case for agroecology as a tool for recovering from this crisis to improve food sovereignty and sustainable food production (Altieri et al., 2020; Gemmill-Herren, 2020; Loker et al., 2020). Despite the pandemic highlighting the need to reorient the UK food system to grow more food sustainably in the UK (Garnett et al., 2020), we are not very hopeful for a significantly stronger level of support for agroecology in the UK, similar to Europe and are concerned that the current UK political and economic climate may not be favourable for the implementation of sustainable farming practices including conservation biological control.

Barriers to overcome for a wider adoption of conservation biological control

Even though the UK policy climate might not be the most favourable, future research will need to address barriers to adoption of conservation biological control (CBC), as even after decades of research, its uptake is still limited with very few incentives for larger scale collaborations (Chaplin-Kramer et al., 2019). Barriers to adoption have been identified by research in different contexts, with some echoing our very limited discussions with sampled farmers. Abdollahzadeh et al. (2017) investigated adoption of CBC in rice systems in Iran and revealed some unique factors impacting adoption, such as gender, education, participation in extension program, participation in local cooperatives as well as the use of family labour. Mkenda et al. (2020) who surveyed 300 farmers in Tanzania reported an important lack of knowledge about natural enemies, with 98.7% of those farmers being completely unaware of natural enemies. Less severe in proportion, Martínez-Sastre et al., (2020) investigating orchards systems in Spain, also reported a lack of knowledge, especially regarding invertebrates, as well as serious misconceptions about biological control. In Europe as well, Zhang et al. (2018a) highlighted the low confidence farmers have in natural enemies compared to pesticides, as well as the need for financial

flexibility to support adoption of CBC. As farmers tend to be risk-averse more than change-averse (Tracy, 2015), the limited economic assessments of CBC is a clear issue, with only a few studies reporting economic benefits (Cullen et al., 2008; Gurr et al., 2016; Naranjo et al., 2015; Onstad et al., 2009). Promoting CBC adoption should include linking this practice to profitability if farmers are to be convinced (Gontijo, 2019; Shields et al., 2019) and more research effort in agroecology should be dedicated to improved economic assessments (see van der Werf, Knudsen and Cederberg, 2020). One last barrier that will need to be address is linked to knowledge about the local populations of natural enemies. If modern agriculture practices led to a serious decline in entomofauna (Firbank et al., 2008; Hallmann et al., 2017; Sánchez-Bayo et al., 2019; Thomas et al., 2004), the lack of adequate natural enemies locally could obviously lead to CBC failure (Tscharntke et al., 2016) and a few local Bioblitz or Irecord enthusiasts cannot provide adequate information for the implementation of a successful CBC strategy. The 25 Years Environment Plan suggests that DEFRA will invest at least £200,000 on soil health metrics (UK Government, 2018) in order to improve our knowledge of British soils. We would argue that investment in ecological surveys and training to assess local biodiversity, as well as the inclusion of monitoring in future agro-environmental schemes, would be truly beneficial in order to paint a realistic and up to date picture of the state of our agroecosystems, a necessary base for any successful CBC strategy.

Co-creation of knowledge and social capital

On-farm biodiversity management research is not always easily translated into actual changes in practice, even if more implementation protocols are becoming available (Gurr et al., 2017; Shields et al., 2019) such as the James Hutton Institute "magic margins"[9], or the Game and Wildlife Trust (GWT) beetle banks[10], while independent biodiversity farming advisors who could facilitate those changes are too few (ScotFWAG, pers.comm). While fundamental ecological research is still required to improve our understanding of agroecosystem functioning, knowledge dissemination and training of practitioners is crucial to move forward. Research output has been translated into precious resource tools such as

[9] www.hutton.ac.uk/news/%E2%80%9Cmagic-margins%E2%80%9D-win-innovation-award-rspb-nature-scotland-awards
https://community.rspb.org.uk/ourwork/b/scotland/posts/magic-marginsbiodi
[10] https://www.gwct.org.uk/farming/advice/sustainable-farming/beetle-banks/

the Biodiversity Function project[11], or Conservation Evidence[12] (Dicks et al., 2016) but if redesign of agroecosystems is required for sustainable intensification (Pretty et al., 2018; Wezel et al., 2014), farmers will require a lot more than quality online resources. This fundamental change in practice will require the collaboration of farmers, researchers, agronomists, economists, policy makers and social scientists (Brévault et al., 2019; Doré et al., 2011; Pretty et al., 2018). However, as collaboration takes time and is expensive, a large effort and financial commitment will be required for successful implementations of those new practices, including conservation biological control (Shields et al., 2019), especially when collaboration at landscape scale is required. Engaging farmers in research, as advocated by MacMillan and Benton (2014), may not be enough for such a shift and we would echo Pretty et al. (2018) by also advocating the need for the creation of agricultural knowledge economies as well as the co-creation with farmers of relevant solutions for sustainable intensification. Precious research on agri-environmental schemes adoption and collaboration at landscape scale revealed the need for a participatory and collaborative approach, facilitating communication, negotiation and feedback in order to induce farmers collaboration (Emery et al., 2012; Prager, 2015; Prager et al., 2012). The barriers to the adoption of AES identified by Emery and Franks (2012) are also valid for the implementation of any agroecological management strategy at a landscape scale as a lack of communication and mutual understanding between farmers, a cultural imperative for independence and timeliness and alternative interpretations of risk amongst farmers would certainly impede any successful implementation. Trust of course also comes into play, the trust farmers have in their neighbours, which seems based in part on their performance as 'good farmers'(Sutherland et al., 2012) but also trust in the reliability of advice. This co-creation of agroecological knowledge and solutions also need to be accompanied by the facilitation of creation of social capital (Prager, 2015; Pretty et al., 2018, 2015), to foster trust and collaboration, especially as social learning has been identified as a central process to support agroecological practices (Cullen et al., 2008). Farm clusters and farmers field labs are, we believe, a great example of how to take sustainable intensification forward. GWT farm clusters[13], the Netherlands' Environmental Cooperatives (van Dijk et al., 2015), the French network of agroecological demonstration farms DEPHY[14](Lechenet et al., 2017) or

[11] https://biodiversityfunction.com/
[12] www.conservationevidence.com
[13] www.farmerclusters.com
[14] https://chambres-agriculture.fr/recherche-innovation/dephy-ecophyto/dephy-ferme/

the more local Innovative Farmers Field labs[15] all provide precious lessons on how to foster collaboration and social learning, while allowing researchers involved to produce quality publications, indispensable for future funding. At an even higher level, initiatives such as la Via Campesina[16], an international network of agroecological farmers, can potentially also teach us valuable lessons on how to upscale further collaboration and dissemination, as global amplification of agroecological practices and fundamental redesign of agroecosystems are required to transition to global sustainable food systems (Altieri et al., 2015; Gliessman, 2016; Pretty et al., 2018).

6.7 General conclusion

As part of Integrated Pest Management, conservation biological control can play a part in sustainable intensification. However, the soil has been largely ignored by this field so far, even when considering the management of root pests. In order to improve overall agroecosystem health and multifunctionality, this large piece of the puzzle should attract more attention. This study has attempted to make a case for the inclusion of the soil in future conservation biological control studies and the development of belowground habitat management strategies. Using a community approach, this study has shown that organic management positively impacts soil biological activity, can lead to enhanced pest suppression at plot level, while enhancing the presence of natural enemies. The presence of natural enemies co-occurring in all pest samples and the ubiquitous resource-consumer links highlighted the potential natural regulation happening in fields despite farmers usually underestimating this service. By including entomopathogens and other microfauna antagonists and assessing their potential impact on pest suppression, this study highlighted the need to include this part of the antagonist community in further research. Including a wider range of organic and conventional practices by surveying commercial farms allowed this study to highlight the overall positive impact on organic managements on soil health and plant growth, as well as the potential to suppress root pests faster than their conventional counterparts. Even though this study's impact is limited by issues of scale, replication and species identification, the novel community approach used along with the original data analysis approach could contribute to the further understanding of ecological

[15] https://www.innovativefarmers.org/welcometoriss/
[16] https://viacampesina.org/en/

dynamics in the context of natural pest regulation. While research can provide fundamental ecological knowledge, collaboration between farmers, agronomists, researchers, and policy makers is required to improve the adoption of conservation biological control, as well as other sustainable intensification practices. Co-creation of relevant knowledge and solutions, as well as fostering social learning and collaboration at landscape scale could contribute to the amplification of agroecological practices and overcome some barriers to adoption. Our aim as agroecology scientists is to participate in the redesign of multifunctional agroecosystems, taking into consideration social-ecological networks as both agroecosystem biodiversity and farming communities need to thrive. We would like to echo Brévault and Clouvel's (2019) vision of successful reconciliation of farming practices and natural regulations, where farming communities move towards adopting locally co-designed practices for the management of ecosystem services, integrated into multifunctional resilient landscapes. But time is short, as highlighted by Hunter *et al.* (2017):

> "Time is short: The annual cycle of planting and harvest gives farmers fewer than 35 chances to transform their production systems by midcentury. Scientists also face a limited number of opportunities to develop and test new production and conservation strategies. As a group of (…) agricultural scientists (…), this is the challenge of our careers. By the time our generation retires, agriculture's 2050 goals must be met"

Annexes

Annex 01 - Soil Dry Weight

Principle of the method

Soil is weighed before and after drying, at least overnight, at 105°C

Materials and apparatus

- Oven at 105°C
- Soil sieved < 4mm
- Aluminium foil trays
- Balance
- Falcon tubes (50 ml) or equivalent

Procedure

- Label and weigh empty foil tray. Record weight of tray.
- Mix soil and add about 30 g to foil tray and re-weigh. Record weight of tray + fresh soil.
- Put tray in oven at 105°C. TAKE CARE, OVEN IS HOT. Leave at least overnight.
- Remove trays to cool, USE OVEN GLOVES.
- When cool re-weigh. Record weight of tray + dry soil
- Transfer soil to plastic container, to keep for further analysis if required.

Calculation

Essentially this is the amount of water in the fresh soil sample divided by the weight of dry soil
Gravimetric soil water content = [fresh soil –dry soil] / dry soil

$$= \frac{[(\text{wt tray+fresh soil}) - (\text{wt tray})] - [(\text{wt tray+dry soil}) - (\text{wt tray})]}{[(\text{wt tray+dry soil}) - (\text{wt tray})]}$$

Annex 02 – Soil pH

Measurement of soil pH in fresh soil

Materials and apparatus

- pH/mV meter
- Plastic beakers (disposable)
- pH Buffer 4.01
- pH Buffer 7.00
- Deionised water
- Combination electrode (with temperature compensation, ATC, probe)
- Glass stirring rods

Procedure

1. Weigh (or scoop) 10g (or 10 ml), in duplicate, sieved (2-4mm), field moist soil into 40ml disposable plastic beakers
2. Dispense 20ml of deionised water into the beaker
3. Stir well with a glass rod and repeat this mixing another 3 times over the next 30 minutes, and just prior to the pH being measured
4. Measure the pH within the next 60min (standard practice but not essential)

pH meter calibration – 2 point

1. With power on press mode until pH mode indicator is displayed
2. Rinse the electrode and place into buffer pH 7.00, stir moderately
3. Press 2nd then cal to begin calibration (date and time of the last calibration will be displayed)
4. When READY is displayed and is 'flashing', this indicates that electrode stability has been achieved
5. Press yes; the buffer value is stored and the meter reading freezes for 3 seconds. The meter will then automatically switch to buffer two (pH 4.01) , indicated by P2 on the display
6. Remove and rinse the electrode and place into buffer pH 4.01, stir moderately
7. When READY is displayed and is 'flashing', press yes
8. Press measure, the meter will then advance to the measure mode (slope of electrode calibration is displayed)
9. Place back into one of the buffers to check the calibration, repeat the above if necessary
10. The pH meter is now ready for use

Notes

In organic soils the pH extraction ratio is 1:5 (or 1:10) this produces enough solution in which pH can be physically measured. Alternatively centrifuge the standard 1:2 extractant (5000rpm) and measure the pH in the supernatant. Measuring pH in water is the closest way to estimate the pH in the soil solution.

Soils that have been recently fertilised (agricultural soils) may require pH measurement using 0.01M $CaCl_2$; with 0.6 pH units added to the value obtained. This is to compensate for an increase in electro-conductivity (increase in the soluble salt) due to the fertiliser. Also, by using 0.01M $CaCl_2$ salt matrix, the effect of the fertiliser on the soil pH measurement is lessened (thereby compensating for the influence of the history of fertiliser application).

Annex 03 – Soil basal respiration (OSU Soil fertility Lab+ SRUC)

Method for the determination of CO_2 respired from air-dried soil that has been rewetted. The method was originally reported by Franzluebbers et al. (1996) and expanded by Franzluebbers et al. (2000). Several methods are available for quantifying evolved CO_2. This procedure facilitates a high-throughput framework, using 50 mL centrifuges tubes and an infrared gas analyzer (IRGA). Soils that are ground to <2 mm are typically used.

Materials and apparatus

Sample Preparation and Reaction

- Analytical balance capable of weighing to two decimal places
- 50 mL disposable polypropylene centrifuge tubes (Falcon tubes)
- 10 mL pipettor and tips
- Falcon tube caps with septa installed and sealed with silicone caulk
- Parafilm

Reading Samples on Spectrophotometer

- Carrier gas tank (100% N2 gas + regulator)
- Air Control Valve (Dwyer RMA-150-VSSV)
- LI-840a infrared gas analyzer (IRGA)
- LI-840a software
- 0.5 mL and 1 mL syringe with needle
- CO2 standard gas tank (1% CO2, 99% N2 + regulator)

Procedure:

A. Sample Preparation:
1. Label 50 mL tubes with unknown sample ID's.
2. Weigh out 20 g (± 0.05) adjusted to reach 60% water holding capacity, of the fresh soil sample in corresponding tube (may be done in advance).
3. Leave tubes uncapped until start of sample reaction.
4. Soil checks should be prepared in the same manner as the unknown soils and serve as laboratory reference samples. It is recommended to pulverize and homogenize a large batch of air-dried soil for long-term use. The soil checks allow for a quality control check across respiration runs performed on different batches and over multiple days.
5. Label 3 empty tubes that contain no soil to use as blanks. These will be used later in calculations.

B. Sample Rewetting and Incubating:
1. Place samples in a fume hood to make sure the initial level of carbon dioxide in each tube is uniform.
2. While keeping the tube under the hood, use the 10 mL pipette to dispense pre-determined volume of deionized water (typically 3 mL, to reach 120% water holding capacity) into each tube. The water should be dispensed in a circular motion to prevent any splashing or disturbance of soil. Keep a timesheet of when the water is added to each sample. This should be done in 1 minute intervals. Be careful not to breathe into sample tube as this will increase the carbon dioxide levels and give false values.
3. Cap the samples while the tube is still under the hood.
4. Wrap parafilm over the cap onto the sides of the tube to seal.
5. The 3 blanks should be treated in the same way without adding soil or water.

6. Place sealed samples in an incubator set to 25oC. Incubate for 24 hours. Record incubation temperature to be used later in calculations.

C. Preparing the IRGA:
1. Turn on gas tank containing IRGA carrier gas (N2) 20-30 minutes prior to sample start time. Set carrier gas flow to 70 mL/min (50-100 mL/min is an acceptable range). Measuring standards typically takes 10 minutes.
2. On the computer connected to the IRGA, open LI-COR software to run LI-840A.
3. Click File > Connect and connect to appropriate port.
4. When flow rate of carrier gas remains constant (typically takes 10 minutes), click:
View > Calibration and calibrate CO2.
5. To start logging measurements, click: Logging > Start. Enter a file name for the data file and save. The CO2 values will be logged at 1 second intervals.

D. Measuring CO2 Standards:
1. There will be 3 volumes of standards taken (1 mL, 0.5 mL, and 0.25 mL).
2. Turn on standard tank (1% CO2, 99% N2).
3. Insert the 1 mL syringe into standard tank septa and draw 1 mL of the CO2 standard.
4. Inject 1 mL quickly into the injection port. Record time of injection. The CO2 concentration will be digitally logged.
5. Repeat steps 3-4 until there are 3 readings with 1 mL. Wait 1 minute between standards, or until the baseline of CO2 returns to zero.
6. Using the 0.5 mL syringe, repeat steps 3-5 with 0.5 mL and with 0.25 mL of the CO2 standard. Note we are manipulating CO2 standard volumes as a proxy for different CO2 concentrations here. This negates the need for 3 different tanks with 3 different CO2 concentrations.
7. The standards should be completed before and after reading unknown sample.

E. Reading Samples on IRGA
1. Remove samples from the incubator and record the temperature.
2. These should be read as close to the 24hr mark as possible, hence the recording of time when water was added.
3. At exactly 24 hours since rewetting began, insert 1 mL syringe into septa in lid of unknown soil sample.
4. Pump syringe 5 times to thoroughly mix air inside the tube.
5. Draw 1 mL of air from the sample tube and insert into the IRGA septa at the minute mark, keeping in time with the time recorded 24hrs earlier.
6. Inject 1 mL quickly into the injection port. Do this in the same manner as the standards (record time and wait for the CO2 level to return to 0).
7. Repeat steps with remaining samples, soil checks and blanks.
8. At the end, repeat the CO2 standard measurements again as described above in section IV.

F. Equipment Clean Up
1. After taking CO2 readings, stop logging to save as a .txt file.
2. Turn off the carrier gas tank and standard tank, making sure to release the pressure.
3. LI-COR recommends leaving the IRGA on all the time.

G. Calculations
1) Identify peaks of standards, blanks and unknown samples:

a. The LiCor software logs CO_2 concentrations every second. Samples injected will produce CO_2 concentrations that increase quickly over time, peak, then decrease back to baseline. The peak, or highest concentration of CO_2 is the value used for each corresponding sample. Peaks that correspond to each standard, blank and unknown need to be identified and all remaining CO_2 concentrations should be eliminated.

b. This can be done manually in excel or automatically using the turnpoints() function in the package pastecs in R.

2) Calculate Adjusted IRGA ppm:

a. Average the 3 blanks to get a baseline IRGA ppm.

b. Subtract the averaged baseline IRGA ppm from each unknown soil sample IRGA ppm value. This step accounts for CO_2 concentration at start of incubation.

3) Calculate a Standard Curve:

a. Average the 3 replicates for each of the 3 standards at the beginning of the readings. Repeat this step with the standards read after the unknown samples.

b. Average the 2 corresponding standards before and after readings in step 3a. Note any systematic drifting of values (increase/decrease) over the course of the readings.

c. Calculate a regression line equation between the known CO_2 gas standards and the empirically-derived gas standard.

i. Y-axis: the known CO_2 gas standards: i) 1 mL: 1% or 10,000 ppm, ii) 0.5 mL: 0.5% or 5,000 ppm and iii) 0.25 mL: 0.25% or 2,500 ppm.

ii. X-axis: the calculated averaged IRGA ppm measurements.

iii. Extract slope and intercept terms from regression line.

4) Calibrate the Adjusted IRGA ppm results to the Standard Curve:

a. Multiply the slope of the regression line by the adjusted IRGA ppm and then add intercept to calibrate to the known ppm.

5) Convert the calibrated and adjusted ppm CO_2 (which is on a volume basis) to μg CO_2-C per L headspace with the below equation (ideal gas law):

a. Cm (μg CO_2-C L headspace-1) = (Cv x M x P) / (R x T), where

i. Cv = ppm (volume) CO_2

ii. M = molecular weight of C (12 μg /μmol)

iii. P = Barometric pressure (1 atm)

iv. R = universal gas constant (0.0820575 L · atm / K · mole)

v. T = incubation temp in °K (273.15 + oC)

6) Convert μg CO_2-C L headspace-1 to μg CO_2-C gram soil-1:

a. Multiply μg CO_2-C L headspace-1 by the volume of the incubation chamber (in L) and divide by the weight of the soil used in the incubation. This is equivalent to the more commonly reported mg CO_2-C kg soil-1.

7) Finally, to convert mg CO_2-C kg soil-1 to a rate, divide by the number of days incubated.

References

Franzluebbers, A.J., R.L. Haney, F.M. Hons, and D.A. Zuberer. 1996a. Determination of microbial biomass and nitrogen mineralization following rewetting of dried soil. Soil Sci. Soc. Am. J. 60:1133–1139.

Franzluebbers, A., R. Haney, C. Honeycutt, H. Schomberg, and F. Hons. 2000. Flush of carbon dioxide following rewetting of dried soil relates to active organic pools. Soil Sci. Soc. Am. J. 64:613–623.

Franzluebbers, A. J. 2016. Should Soil Testing Services Measure Soil Biological Activity? Agric. Environ. Lett. 1:150009. doi:10.2134/ael2015.11.0009

Haney, R.L., Haney, E.B. 2010. Simple and rapid laboratory method for rewetting dry soil for incubations. Communications in Soil Science and Plant Analysis. 41(12):1493-1501.

Wade, J., Culman, S.W., Hurisso, T.T., Miller, R.O., Baker, L., Horwath, W.R. 2018. Sources of Variability that Compromise Mineralizable Carbon as a Soil Health Indicator. Soil Sci. Soc. Am. J. 82:243-252.

Annex 04 – Community-level physiological profile using Biolog EcoPlates ©

Principle of the method

This method is based on the incubation of soil suspension into a Biolog Ecoplate at 15°C over 6 days and the daily measurement of the OD595 of each plate well by a microplate reader, in order to determine the area under curve (AUC) for each carbon source and the average well colour development (AWCD) for each soil sample.

Materials and apparatus

- Incubator at 15°C
- Soil sieve 4mm
- Shaking apparatus
- Filter paper (N-free)
- Falcon tubes (50 ml)
- Plastic sample storage bottle
- Balance
- Biolog Ecoplate
- Microplate reader

Chemicals and solution

¼ strength Ringer's solution (2.25g NaCl, 0.105g KCl, 0.12g CaCl2.2H2O, 0.05g NaHCO3 l-1)

Procedure

- if soil was stored at 4°C, incubate at 15°C for a week.
- Weigh 50g of fresh soil
- Sieve through 4mm sieve
- Weigh 5g of soil, place into 50mL Falcon tube with 20mL ¼ strength Ringer's solution
- Shake for 30min
- Adjust soil suspension for OD600=0.2
- Inoculate Biolog Ecoplate with 15mL of adjusted soil suspension
- Read OD595 straight away of whole plate with microplate reader (t=0)
- Incubate plate in the dark at 15°C (keep moist paper towel in incubator)
- Measure OD595 of whole plate every day for 6 days

Calculation

To facilitate the extraction of all soil samples data and avoid manipulation errors, a dedicated parser was coded using Python (Keir Lawson, 2013)
All well measurements values were reset by subtracting their OD595 at t=0

- Area under curve is calculated using an approximation of the surface underneath the OD595 curve over time for each well:

$$A_{ik}=1/2 \sum_{j=1}^{n-1} [(t_{j+1}-t_j)(C_{ikt(j+1)}+C_{ikt(j)})]$$

- Average well colour development= simple average of OD595 of all wells at chosen time

References

Hackett, C. A., & Griffiths, B. S. (1997). Statistical analysis of the time-course of biolog substrate utilization. Journal of Microbiological Methods, 30(1), 63–69. https://doi.org/10.1016/S0167-7012(97)00045-6

Annex 05 – Extraction of free- living nematodes

Testing soil for presence of free living nematodes

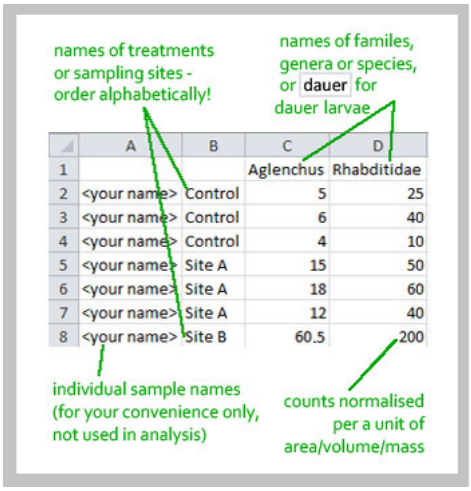
Procedure

On arrival
1.1 Samples sent to the Crop Clinic are logged in and given a unique Crop Clinic reference code. At all times samples are handled with care as nematodes can be killed by rough handling and the analysis is only for living nematodes.
1.2 Samples are secured by checking seals on bags and placed in a box labelled with sample reference numbers for transferral to the soil wash facilities cold storage room.

2.Extraction of nematodes
2.1 At all times during the analysis equipment and work surfaces must be kept cleaned to avoid cross-contamination
2.2 Remove sample from cold storage and thoroughly mix before weighing out a 250g subsample into a 1litre plastic beaker.
2.3 Add about 600ml of water to the beaker and gently crumble the soil, gentle handling avoids damaging the nematodes. Allow to stand for 30 mins (light soils) or 60 mins (heavy soils)
2.4 Wash the contents of the beaker through a 1.0mm aperture sieve into a 5 litre plastic bucket (removes larger stones and debris) and top up with water.
2.5 Mix the soil suspension in the bucket thoroughly by hand and after 20 seconds sedimentation time carefully decant the supernatant fluid through a 150µm aperture sieve into another 5 litre bucket. Discard the sediment at the bottom of the bucket
2.6 Using a gentle spray wash the material caught in the sieve into a beaker (A).
2.7 Agitate the suspension collected in the bucket which has passed through the 150µm sieve. Allow to stand for 20-30 seconds then decant through a 63µm sieve into a bucket. Discard the residue at the bottom of the bucket.
2.8 Wash the material caught in the sieve into a beaker (B)
2.9 With the remaining suspension in the bucket which has passed through the 63µm sieve repeat steps 2.7 and 2.8`but decant through a 53µm aperture sieve with the sieve washing also going into beaker (B).
2.10 Pour the contents of beaker (A) onto a 95µm mesh nylon sieve and place into a 15cm diameter Baermann funnel which is filled with sufficient water to submerge the debris.
2.11 Pour the contents of beaker (B) onto a Kleenex 2ply tissue which is supported on a coarse nylon sieve and place in a Baermann funnel as previously described in 2.10
2.12 After 48 hours collect the nematodes by drawing off water from the funnels into 3" x 1" glass tubes. The collection from beaker (A) should mainly contain the larger nematodes such as Longidorids while the collection from beaker (B) will contain the smaller nematodes such as Trichodorids and Pratylenchids.

3. Nematode identification and enumeration
3.1 Empty the contents of the tubes into Doncaster counting dishes and use a suitable stereo zoom microscope to identify and count the relevant nematode species.
3.2 Results are expressed as the number of nematodes per 250g of soil.

4. Further analysis, using NINJA method
4.1. Format your data using the template below



	A	B	C	D
1			Aglenchus	Rhabditidae
2	<your name>	Control	5	25
3	<your name>	Control	6	40
4	<your name>	Control	4	10
5	<your name>	Site A	15	50
6	<your name>	Site A	18	60
7	<your name>	Site A	12	40
8	<your name>	Site B	60.5	200

4.2. Save in usual Excel format and upload here https://sieriebriennikov.shinyapps.io/ninja/

Annex 06 – Soil Hot water extractable carbon (from Ghani et al., 2003)

Principle of the method

There is a strong correlation between HWC and other biochemical measures, so HWC serves as on integrated measure of soil quality. This method is based on incubation of waterlogged soil overnight at 80°C. At the end of the incubation, accumulated extractable carbon is measured. Ideally soils were sieved <4mm in the field then stored at 4oC.

Materials and apparatus

- Water bath at 80°C
- Soil sieved < 4mm
- Vacuum filtration equipment
- Shaking apparatus
- Filter paper (N-free)
- Membrane filters 0.45 um
- Falcon tubes (50 ml)
- Plastic sample storage bottle
- Balance
- Vortex mixer

Chemicals and solution

- Distilled water

Procedure

- Weigh out 3g fresh soil (between 3.0 and 3.5g but record fresh weight) into labelled Falcon tubes. Add 30 ml distilled water and shake horizontally on rotary shaker for 30 mins at room temperature.
- Centrifuge, 5000 rpm for 15 mins, and then decant supernatant to a new, labelled, tube.
- Add 30 ml distilled water to the remaining soil pellet, vortex to mix the soil suspension, and incubate in water bath at 80oC for 16hrs.
- Supernatant from cold water extraction is filtered through 0.45um mesh, TAKE CARE TO AVOID BACK VACUUM SUCKING BACK TAP WATER INTO SAMPLE, and about 20ml of the supernatant is transferred into a labelled (i.e. sample name/ cold/ date) 30ml plastic scintillation vial. Extracts are then frozen prior to analysis.
- After incubation overnight, vortex tubes to mix, centrifuge, 5000 rpm for 15 mins, and then decant supernatant to a new, labelled, tube. Supernatant from hot water extraction is filtered through 0.45um mesh, TAKE CARE TO AVOID BACK VACUUM SUCKING BACK TAP WATER INTO SAMPLE, and about 20ml of the supernatant is transferred into a labelled (i.e. sample name/ hot/ date) 30ml plastic scintillation vial. Extracts are then frozen prior to analysis.
- For each run use a 'reagent blank', add 30ml distilled water to an empty tube and extract and incubate as above for both 'cold' and 'hot' extractions.

Copy of protocol from paper

Soil samples (equivalent 3 g oven dry weight) were weighed into 50 ml polypropylene centrifuge tubes. These were extracted with 30 ml of distilled water for 30 min on an end-over-end shaker at 30 rpm and at 20 °C, centrifuged for 20 min at 3500 rpm and all the supernatant from was filtered through 0.45 μm cellulose nitrate membrane filter into separate vials for carbon analysis. This fraction of the soil organic carbon was classified as water soluble C (WSC). A further 30 ml of distilled water was added to the sediments in the same tubes. These tubes were shaken on a vortex shaker for 10 s to suspend the soil in the water. The tubes were capped and left for 16 h in a hot-water bath at 80 °C. At the end of the extraction period, each tube was shaken for 10 s on a vortex shaker to ensure that HWC released from the SOM was fully suspended in the extraction medium. These tubes were then centrifuged for 20 min at 3500 rpm. The supernatants were filtered through 0.45 μm cellulose nitrate membrane filters.

Annex 07 – KCl Extract of Soils for NH_4-N and NO_3-N

Purpose & Scope

KCl Extract of Soils for NH4-N and NO3-N

Procedure

Check the relevant COSHH document prior to starting this procedure

1.Remove stones from freshly sampled moist soil. Use a ¼" (5.6 mm sieve) if required.
2.10 g of the moist soil is weighed into a 150 ml shaker bottle and 50 ml of 1M KCl is added. The shaker bottle is tightly lidded and placed on an end-over-end shaker for 2 hours. If possible shake immediately after weighing, otherwise store tightly capped under refrigeration.
3.After shaking, filter through a Whatman No. 40 fluted filter paper (150 mm diameter), which has been washed using a 25 ml aliquot of 0.25M KCl immediately prior to filtration of the soil extract solution. The extract is collected into a freezeproof airtight polythene bottle. A 50 ml sample of 1M KCl is also filtered providing a blank for analysis.
4.The extract can be preserved in the deepfreeze at this stage.
5.A soil moisture determination is carried out using 30 - 35 g of fresh soil, recording the exact weight, drying in the oven at 105°C overnight, and recording the exact weight of dried sample after cooling.
6.The KCl extract is then analysed routinely for NH4-N and NO3-N on the auto analyser using a range of standards made up in 1M KCl (a top standard of 2 ppm dropping to a bottom standard of 0.4 ppm are normally employed at this stage). Refer to SOPs CS/ORG/01, CS/ORG/08 & CS/ORG/018.

Note: If the results are outwith the standards range, they may need to be diluted using 1M KCl solution. When sourcing KCl a grade of > 99.5% purity must be used.

Calculations

Using the results from the auto analyzer and the moisture content, results can be expressed as mg/kg soil (OD) i.e. ppm (OD).

1.Determination of Soil Moisture expressed as % of OD soil

Weight of container W1
Weight of container + fresh soil W2
Weight of container + OD soil W3

Weight of OD soil W 3 - W1

Weight of moisture W 2 - W3

% moisture of OD soil (W2 - W3)/(W2 - W1) x 100%

2.Dry weight of extracted soil used can be calculated using the weight of the moist extracted soil (10g) and % moisture of that sample calculated as shown above.

i.e. Moist weight - %moisture = Dry weight
e.g. 10g – 20% = 8g

Analysis of the extracted filtrate solution results in ppm N - NO3 and ppm N - NH4 values, these can be related back to concentrations in the original sample thus:-

i.e. 50 ml of extract from 10 g of moist soil or equivalent of OD weight as calculated from 2 above.

N = X µg x 50 (= X µg is ppm N in OD weight of 10 g moist soil)

N = X x 50/ OD weight µg /g i.e. mg/kg

Annex 07 bis Operation of the Skalar San++

Operation of the Skalar San++ located in laboratory 208 in the Peter Wilson Building KB.

This SOP relates to the Skalar San++ as it is currently located in laboratory 208. It is designed as a guide to provide users with a series of instructions that should allow them to safely turn on, operate and leave the machine fit for the next user. It DOES NOT replace the need for training on the machine prior to operation nor does it propose to cover all potential end uses of the machine. It is recommended that all users familiarise themselves with the machines operations, maintenance and chemistry guide.
Use of the Skalar San++ for functions beyond the basic soil and water analysis, covered in the user manual and routinely conducted by the SAC, should be attempted only once a user is familiar with the system and set up in consultation with the other primary users (at the time of writing these being Oliver Knox, John Parker, Phillip Maskell and Helen Gordon)

Procedure
Turning on of the chemical deck, auto sampler, interface and PC system

- Book the use of the system in advance, via the booking year planner located near the machine in laboratory 208.
- Ensure that the required reagents, solutes and indicators are present in sufficient quantities for the chemistries to be undertaken in the analysis.
    - These can be prepared in advance in accordance with the manufacturers details as given in the Skalar methodologies provided with the system.
    - Generally responsibility should lie with the next user to make sure they have sufficient solutions for their run. However, if periods of high use are occurring (i.e. daily runs) then please be courteous and liaise with other users as to where responsibility for solution provision lies.
    - DO NOT make up fresh solutions mid run as this is likely to affect results and QA.
    - Most solutions are stored in the fridge, but all have a limited shelf life. If the machine and the desired chemistry have been unused for more than 2 weeks then consider refreshing all solutions for the particular analysis.
- Place the required solutions within reach of the solution feed lines (i.e. around the auto sampler platform).
- Remove the required solution feed lines from the wash and storage water containers and place them in the appropriate chemistries.
    - NOTE: The phosphate lines (GREEN tags) are kept separate for washing and storage from the nitrogen based systems lines to prevent contamination.
    - Ensure the glass weight reaches the bottom of the solutions.
    - Avoid mixing of lids with solution bottles.
- Check and install the plattens (Plattens are the top parts of the pumps that drive the solutions through the system).
    - Prior to fitting the platten to the pump housing smear the surface with a minimal quantity of grease, usually recovered from the platten surface.
    - Ensure the top black tension lever is pointing upright.
    - Engage the rear screw pins into the housing mounts located on the inboard side of the San++.
        - Ensure that none of the tubes become trapped or lifted from their guides as this happens.

    - Some gentle giggling (try applying gentle pressure to one side more so than the other) of the platten might be required to ensure it fits in properly.
  - Push down the outboard side of the platten (nearest to the auto sampler) whilst holding in the front levers (two on each platten) to retract the side mounted smooth pins. These pins should engage with holes in the pump housing side and the levers should return to their original position if the platten is correctly fitted.
  - Push down the black tension levers on top of the platten.
- Turn on the Skalar San++ using the GREEN 'Power' button located on the front panel of the large chemistry deck.
- Ensure that the running speed of the deck is set at normal.
  - If not, then depress the SILVER 'Speed Control' button to cycle through the deck's four speed capabilities (Stop, Slow, Normal and High) as indicated by a RED & GREEN lights next to the printed wording.
- If TOTAL NITROGEN is being assessed, turn on the UV digester and the backpressure unit.
  - The UV digester is activated by switching the GREEN switch on the black control box, which is located between the chemistry deck and the tower integrator and on top of the pump control.
  - The backpressure unit pump is turned on using the GREEN 'Power' button on the left of the control box, located under the UV digester control unit.
  - At the back left of the chemistry deck is the air-liquid separator column. With the backpressure unit on the 3 way valve at the top of this column needs to be switched so the RED DOTS are aligned with the 'Chemistry' and 'Pump' labeled lines.
  - After 15 minutes ensure the backpressure is at 0.35 – 0.5 bar on the dial on the right of the backpressure controller. If not adjust the pressure via the valve knob located at the rear of the controller unit.
- If TOTAL NITROGEN or NITRATE/NITRITE is being accessed then the cadmium columns (located on the chemistry deck) need to be opened once there is buffer flow within the system (~ 5 to 10 minutes after pump activation).
  - On each of the columns is a SILVER mechanical switch marked with two double arrows
  - Rotate the SILVER switch through 90o so that the arrows make a continuous circuit between the glass cadmium column housing and the chemistry side of the deck.
  - As you look at the machine from the lab the arrow orientation would therefore be as follows: columns off columns open to buffer flow
  - Black marks have been placed on the switch and tubing anchor on the chemistry deck. If these are aligned then the columns are open.
- Turn on the decks heating systems IF NEEDED.
  - The heating control panel located at the front of the machine turns on the 40oC heaters needed for PHOSPHATE and AMMONIA analysis.
  - The heating control panel at the rear of the deck (nearest the wall) is needed for TOTAL NITROGEN and should be set at 107oC
  - The ON button looks like a double circle (⓪) at the left of the control display.
  - Once on, the displays on both panels show the current temperature of the heating system. If not on then the display reads 'OFF'
  - The set temperature can be checked by depressing the relevant 'S1' or 'S2' button briefly. Holding them for longer and using the up and down arrows (▲, ▼) allows the temperature to be reset.
- Turn on the auto sampler via the flick switch located at the back of the unit.

- It appears to BE ESSENTIAL to turn on the auto sampler deck prior to the computer, otherwise it is not detected.
- When turning on the auto sampler platform ensure that the wash line is in either water or the correct solution for the chemical analysis being undertaken (i.e. deionised water, KCl or K2SO4).
- Ensure that there is sufficient to complete the run.
- Make sure that there is a flow of solution through the needle by looking for droplets emerging at the sample needle tip and the movement of solution and air around the auto sampler pump. If there IS NOT then check the inlet on the side of the needle as this appears to regularly block.

- Turn on the tower integrator using the SILVER button located on the front of the display panel.
  - Once on, wait for the display panel above the SILVER on switch to display 'SKALAR ANALYTICAL' as one of its flash messages before proceeding.
- Turn on the PC and monitor.

Preparing for analysis and using the software

- With the PC on, double click the 'Flow Access' desk top icon.
- Press 'Ctrl' and 'F12' to bypass the security login in screen.
- Double click the 'Active System' icon
- From the 'Analyse- Select System' pop up window, select 'Open' for the highlighted SAC option.
- The following screen (Figure 1) shot should appear:

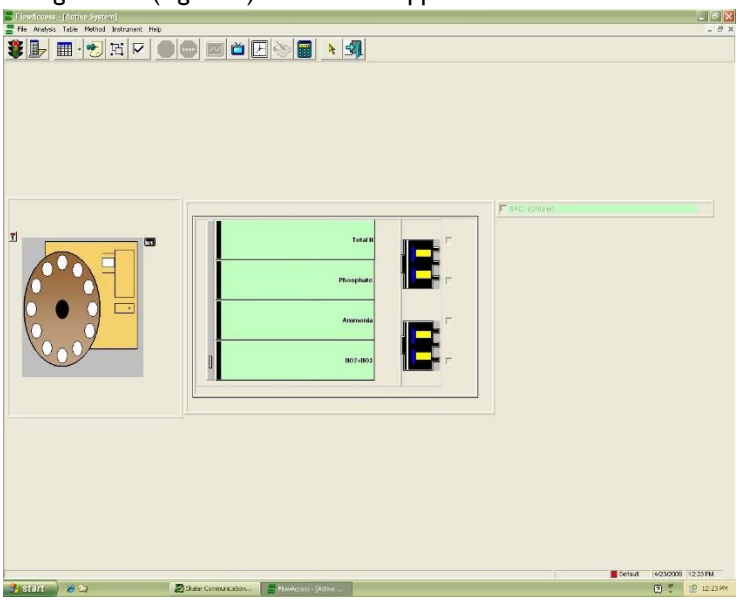


Figure 1 Screen shot of system schematic and selection icons

- The important areas of this screen shot are highlighted below in Figure :

Figure 2 Screen shot of selection icons toolbar

- The options/icons presented on this toolbar are from left to right
  - Traffic light – a system indicator
  - Control panel – useful for running test runs
  - Table
  - Methods
  - ?
  - Analysis selection
  - Start (currently not shown as no table loaded)
  - Stop – used to stop each stage of the analyzer
  - Real time display – takes you straight to real time display upon selecting
  - ? TV
  - ? Clock
  - Notes
  - Results – takes you straight to the results screen
  - Selector
  - Exit
- For most of these they are operated by selecting the icon with a left click.
  - The mouse changes to the form of the icon and can then be used to select that function on the screen display of the auto sampler or the chemical deck.
- The most important of these functions to know of are currently:
  - Analysis selection.
    - Left click on the 'Analysis selection' icon that looks like a tick in a white box.
    - Move the changed mouse pointer over the desired chemistry selection box, all located at the far right of the schematic and in line with the GREEN blocks for each chemistry.
    - Click in the box, which results in a tick appearing in it.
    - This chemistry will now be assessed by the system in the subsequent run.
  - Method
    - Left click on the 'Method' icon
    - Take the mouse down to the chemistry deck side of the on screen schematic.
    - With the mouse pointer (now with a 'pin' appearance) over the GREEN blocks, representing one of the potential chemistries for Total N, Phosphate, Ammonia and NO_3&NO_2, left click again.
    - This opens the pop up window for the selected methodology.
    - A series of options are available here, but it is currently most commonly used for checking the values of the standards for a particular methodology.
  - Table
    - Upon selecting the table icon a pop up window appears
    - Select the 'Remove table' option at the bottom of the list and click the 'OK' button.
      - Existing tables are not lost; they are just removed from the operating system.
      - It is possible, if you are a returning user to go straight to the edit table function here.
    - When prompted select 'Yes' you do want to remove the current table.
    - Return to the table icon and select again with a left click.
    - 'Create new table' is highlighted in the pop up window, so click the 'OK' button.

- On the next screen the 'Create sample table (attach)' top option is highlighted, so click the 'OK' button
- A carousel window appears as shown in Figure.
- The tracer, standards, drifts, washes and unknowns can be entered into this schematic carousel, but many users find the table format easier.
- To access the table format, select the 'View table' button located in the panel in the middle of the screen.
- An Excel based window appears as seen in Figure .
- The table can be populated in the 'type' column with the normal series to start a run.
- This is:
    - Tracer (highest standard), Drift (second highest standard), Wash, six Standard solutions, Drift, Wash, twelve Unknowns, Drift, Wash, twelve Unknowns….
    - Entering the underlined first letter brings up the indicated description, but check it is the right one before hitting 'Enter' to accept.
    - Adding a Drift automatically causes a Wash to follow
- Once a table is prepared the 'Position' column has to be filled before it can be saved.
    - Positions do not correspond to the GREY numbering in the first column of the screen.
    - Positions can be auto filled as in Excel with selecting the last entry, dragging down, right clicking and opting for the 'Auto Number' option. See Figure .
    - 'Auto Number' can also be used on samples with sequential numbering.

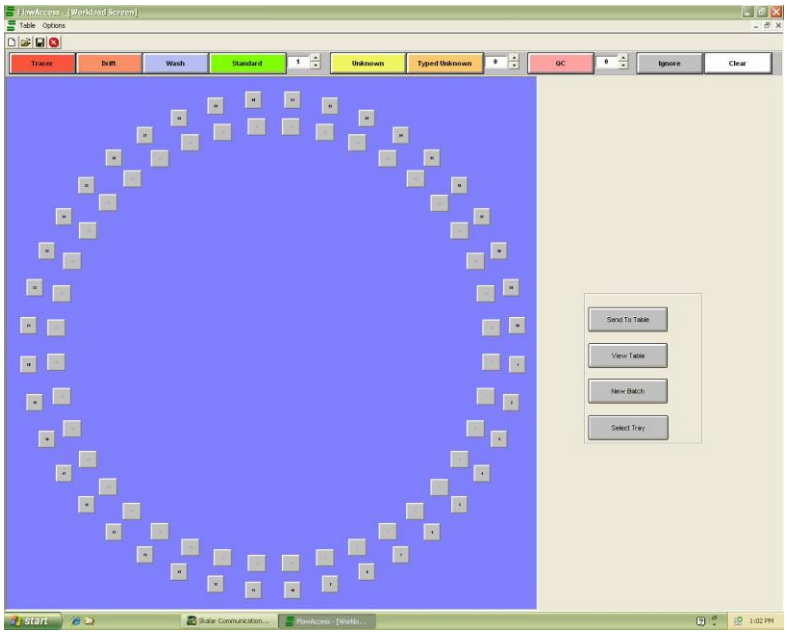


Figure 3 Auto sampler screen schematic. 'View Table' button on right hand side half way down screen

- Save the file in a suitable directory with an appropriate name.

- This returns to the main screen (**Error! Reference source not found.**) and the 'Start' icon should now be green.
- Load the samples (Unknowns) and standards into the outer ring of the Auto Sampler carousel (when running with only 1 needle) according to the position indicated in the table.
    - You can return to view the table using the 'Table' icon and then the 'Edit Table' command on the pop up window.
    - Once the sampler is running you can not change any data on the table 5 positions beyond the current sample being drawn up. This can allow for sample dilutions to be included if needed.
    - It is possible to edit table data in 'Post analysis' after a run should mistakes be discovered.
- Select the 'Start' icon and the sampler should initiate, the auto sampler should indicate it is 'Running' and no longer in 'Stand by' and the 'Real time' view should start to develop a 'time' and 'reading' signature.
- After the standards have all been sampled and analysed through the 'Results' icon can be used to evaluate the run and the analysis.

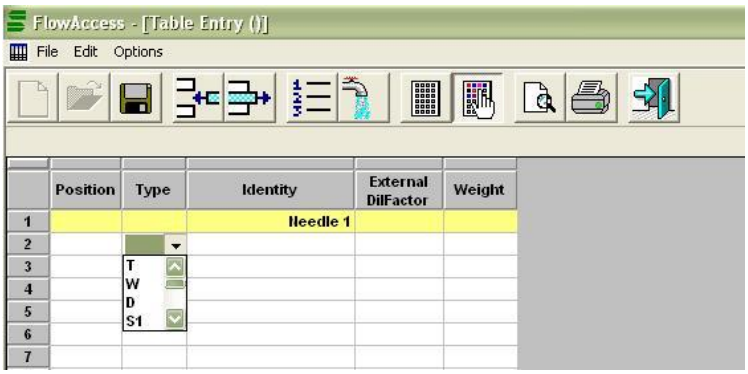


Figure 4 Table screen shot with some of the 'Type' drop down or first letter entries shown.

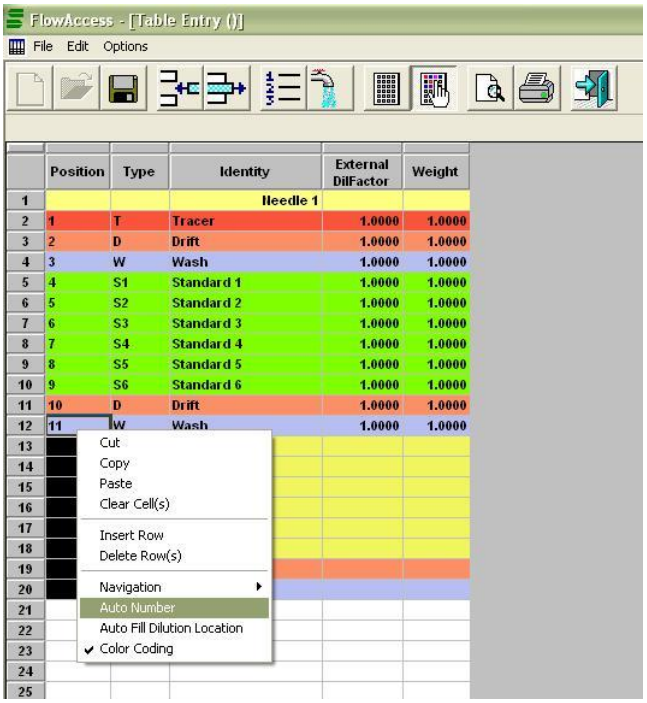


Figure 5 standard table to which the positions are being added.

- Control Panel
  - Left click on the 'Control panel' icon
  - Drag the mouse over the sampler wheel on the on screen schematic (on left in previous screen shot).
  - Left click on this
  - Select the 'Test' function half way down the pop up window.
  - Enter a 'Cup number'
  - Click 'Go' then 'OK' buttons.
  - Move the mouse over the Real time display icon and click on it.
  - The sampler will now sample and run a test on the selected cup allowing a check of the systems operation prior to commencing a run.

Switching off the machine

Upon completion of the sample run the machine needs to be washed through with water and turned off. To do this the following procedures is intended as a guideline.

- Switch off the temperature controllers by depressing their ON buttons (⓪) again. The display should read off.
- Turn off the UV digester via the GREEN switch on the controller box if it was in use.
- Return the SILVER mechanical switches on the cadmium columns to the closed position so that the column is separated from the remainder of the chemistry deck prior to washing (i.e. the columns remain buffer filled).
- Remove the solution and reagent lines and place them in the appropriate deionised water wash solutions.
    - REMEMBER keep the Phosphate lines separate
    - It is worth quickly rinsing the ends of the lines with deionised water before placing into the wash solutions. This reduces the risk of cross contamination and minimizes the chances of any thing growing in the wash solution if the machine is not in use for prolonged periods.
- Run the wash solution through the chemistry deck for 30 to 45 minutes.
- Turn off the chemistry deck with the GREEN button on the front panel.
- Switch the pressure valve on the liquid-air separator column at the back left of the chemistry deck so the RED dots align with 'Waste' and 'Chemistry'.
- Turn off the backpressure unit via the GREEN switch on its left hand side of the front panel.
- Lift the top mounted tension levers on the platten and remove them from the pump deck housing by pushing in the front levers, dislodging the rear screwed holding bolts and lifting them clear.
- Turn off the auto sampler with the switch at the back.
- Turn off the interface with the SILVER switch on the front.
- Turn off the computer and monitor once analysis and file saving is complete.

Annex 08 – Soil organic matter – Loss on ignition LOI

Purpose

The determination of the mass loss on ignition of agricultural soil and determination of Total Ash for other materials such as animal feeds, plants, organic waste and other agricultural samples.

This method can be used for animal feeds, organic wastes, plants and agricultural soil as well other agricultural materials. The method for agricultural soil, animal feeds, organic wastes and plants requires dehydrating at 100°C ± 5°C for three hours and ashing at 550°C ± 25°C taking a total of 2 hours and 15 minutes (includes cooling period).

LOI - Air dried soil is dehydrated and then ashed, and the loss on ignition expressed as a percentage of the dehydrated sample.

ASH/DM60 – Non-soil samples are expressed as total ash g/Kg of sample remaining after the destruction of organic matter contained within the sample material (plant, organic waste, animal feeds and other agricultural materials).

Safety

The COSHH and Risk Assessment forms for this procedure should be familiar to the operator before commencing any work. If not, consult them before undertaking any work associated with this document. Suitable protective clothing should be worn when conducting this procedure.

Material

- Metal trays
- Porcelain crucibles
- Pyro Advanced Microwave Muffle Furnace
- Calibrated Gallenkamp Hotbox Oven
- Four-place electronic balance plus PC with Labware LIMS for direct data capture.

Procedure

Sample Preparation Procedure
Refer to SOP/VS/CHEMPRECEIPT and SOP/VS/CHEMPSOILPREP.
Worksheet Preparation
Refer to User Guide/ASD/30 Labware LIMS Batching and Analysing Wet Chemistry.

ASH, DM60, LOI Analysis

- Open the relevant worksheet in LIMS and ensure data capture is switched on along with the balance being used (if needed).
- Set out the number of porcelain crucibles required in accordance to the worksheet on a metal tray.
- Tare the balance so that the reading is zero and place a porcelain dish on the balance.

- Press the print button to record the weight in the W1 column.
- Place approximately 7 – 10 g for soil and 3 – 5 g for animal feeds in the foil dish and press print to record the weight in the W2 column. Smaller weights can be used if necessary, especially for some animal feeds samples; e.g., Hay which can be very light or molasses which can be volatile at high temperatures.
- Place the tray of samples in the Gallenkamp Hotbox set at 100°C ± 5°C for a minimum time of three hours.
- Remove the samples from the 100°C oven and allow samples at least 30 minutes to come to room temperature in a desiccator.
- Re-open the worksheet in LIMS, ensure data capture is switched on along with the balance being used (if needed).
- Tare the balance and weigh the samples into the W3 column. This will calculate the DM60 (dry matter content) results for samples.
- Place the samples in the microwave muffle furnace set at 550°C ± 25°C and use the method set up for ASH and LOI (this takes a total of 2 hours 15 minutes including the cooling period).
- After the ashing has completed, switch off the microwave muffle furnace and open the door, carefully take the samples out in the correct arrangement according to the worksheet/tray position.
- Once the samples have cooled sufficiently in a desiccator (approximately 15 minutes) they can be weighed back.
- Open the worksheet in LIMS, ensure data capture is switched on along with the balance being used (if needed).
- Tare the balance and weigh the samples into the W4 column.
- The LOI for soils and ASH for other samples are calculated automatically once W4 has been entered.

Annex 09 – Soil Potentially mineralizable N

(from Canali and Benedetti, 2006)

Principle of the method

This method is based on incubation of waterlogged soil for 7 days at 40°C. At the end of the incubation, accumulated ammonium is measured. Ideally soils were sieved <4mm in the field then stored at 4oC.

Materials and apparatus

- Incubator at 40°C
- Soil sieved < 4mm
- Shaking apparatus
- Filter paper (N-free)
- Falcon tubes (50 ml)
- Plastic sample storage bottle
- Balance

Chemicals and solution

- 2 M KCl solution: dissolve 149 g of KCl in 750 ml of distilled water in a 1000 ml glass flask; bring up to volume (1000ml) with distilled water.
- Distilled water

Procedure

- Weigh out 2 x 10g fresh soil (between 9.75 and 10.25g but record fresh weight) into labelled Falcon tubes. Put lid on one tube (control) and place back at 4oC.
- To the other tube (test) add 20 ml distilled water, close the tube and then shake manually until the soil is completely suspended.
- Incubate the (test) tube for 7 days at 40°C. During the incubation, re-suspend the soil regularly (i.e. each morning or evening, but not at weekend) by manual shaking.
- After the incubation, take the (control) and (test) tubes, add 20 ml distilled water TO THE CONTROL TUBE ONLY.
- Add 20 ml 2M KCl solution to both tubes.
- For each run use a 'reagent blank', add 20ml distilled water and 20ml 2M KCl to an empty tube and extract as below.
- Place the tubes horizontally on an orbital shaker and slowly increase the speed to 120 rpm or until the soil is in suspension. Shake for 30min. Centrifuge tubes at 5000rpm for 15mins. About 25ml of the supernatant is transferred into a labelled 30ml plastic scintillation vial. Both the extracts from the un-fumigated and fumigated soils are then frozen prior to analysis.
- Calculation
- Mineralized nitrogen during 7 days of incubation is calculated by subtracting the ammonium measured (□g NH4+-N/g soil) in the sample that was not incubated from that measured in the incubated sample.
- In order to verify that anaerobic conditions occurred during incubation, the nitrate and the nitrite presence should be assessed - only traces of NO3--N and NO2--N should be found.

References

Canali, S., Benedetti, A., 2006. Soil nitrogen mineralization. In: Bloem J., Hopkins, D.W., Benedetti, A. (Eds), Microbiological Methods for Assessing Soil Quality, CABI, Wallingford, UK, pp. 127-135.

Keeney, D. R., and Nelson, D. W. 1982. Nitrogen-inorganic forms. In "Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties" (A. L. Page, R. H. Miller, and D. R. Keeney, Eds.), pp. 643–698. American Society of Agronomy, Madison, WI.

Annex 10 – Soil microbial biomass

Purpose & Scope

Determination of the Microbial Biomass Carbon (MBC) in soil by determining the difference in the Dissolved Organic Carbon (DOC) concentration between Fumigated and Un-fumigated soil.

Equipment

- Vacuum Oven
- Vacuum Pump
- Orbital Shaker
- 250ml plastic jars, 120 glass jars
- Chloroform (25ppm Amylene)
- Soda Lime
- KCl
- K2SO4
- Anti-bump granules

Procedure

Sample Preparation:

Weight out 4 to 6 replicate samples of 15g sieved (2-4mm), mixed, fresh soil. The 2 /3 replicates to be fumigated are weighed into 120ml glass jars; the remaining 2 / 3 replicates to be un-fumigated are weighed into 250ml plastic jars. Additional replicate jars are required for blanks.

Fumigation:

1. Place the glass jars into a vacuum oven, which has moistened tissue layering the shelves, then place a 25ml vial of soda lime, and a 50ml beaker containing 30-40ml of Chloroform (stabilized with 25ppm amylene) and 2-3 anti-bump granules.
2. Evacuate the oven until the chloroform is seen to boil vigorously, continue for several more minutes, before sealing the oven
3. Ideally place the oven in a constant temperature environment of 25oC, in the dark for 24hrs. After this period check that the vacuum has been maintained and that most, but not all of the chloroform has been introduced into the fumigation system
4. Remove the jars and place into a running fume cupboard, discard the tissue, recycle the soda lime and remaining chloroform. Wipe down the inside of the oven then replace the jars, close and evacuate for 3-4 minutes. Break the vacuum, place the jars into a running fume cupboard for 1-2 minutes and repeat this procedure until there is no detectable smell of chloroform from the samples (usually 6+ cycles). Please note that samples that contain a high level of organic matter e.g. straw may require the direct
application of 1-2ml of chloroform to them just prior to stage 1 of the Fumigation procedure.

Please note:

The Un-fumigated replicate soil samples are extracted at the start of the fumigation period

Extraction with 0.5M K2SO4 or 2M KCl:

1. In this method Un-fumigated and Fumigated soil samples are extracted in a ratio of 1:4, with 15g of fresh
soil extracted with 60ml of 0.5M K2SO4 or 2M KCl plus the replicate blank containers.
2. Place the jars on an orbital shaker and slowly increase the speed to 120 rpm or until the soil is in suspension. Shake for 30min

3. Remove the jars from the shaker and allow the soil to settle for several minutes after which the supernatant is transferred to a 50ml centrifuge tube and spun at 5000rpm for 15mins. About 25ml of the supernatant is transferred into a 30ml plastic scintillation vial. Both the extracts from the un-fumigated and fumigated soils are then frozen prior to analysis.
(Note that a white precipitate of CaSO4 usually forms)

Analysis:
If 0.5M K2SO4 is used as an extractant then the Dissolved Organic Carbon can be determined using the Rosemount-Dohrmann DC-80 **or** the Flash 2000 Organic Elemental Analyser. If 2M KCl is used as an extractant then **only** the Flash 2000 Organic Elemental Analyser can be used for the DOC determination.

Total Organic Carbon analyzer DC- 80 (see method of analysis SJP).

Microbial biomass extracts are diluted 1 + 1 with Sodium Hexameta-Phosphate (5%) pH'd to 2.1 using Ortho-Phosphoric acid prior to analysis.

Calculation
(Fumigated DOC – NonFumigated DOC)/ 0.45 = μg g^{-1} MBC
or
(Fumigated DOC – NonFumigated DOC) * 2.22 = μg g^{-1} MBC

Annex 11 - Example of replicated randomised block design for our inoculation experiments

101	conventional	belstar	no_eggs
102	conventional	belstar	eggs
103	sterile	fiesta	no_eggs
104	sterile	fiesta	eggs
105	sterile	belstar	no_eggs
106	organic	belstar	no_eggs
107	organic	fiesta	eggs
108	organic	belstar	eggs
109	conventional	fiesta	eggs
110	organic	fiesta	no_eggs
111	sterile	belstar	eggs
112	conventional	fiesta	no_eggs

201	conventional	belstar	eggs
202	organic	belstar	eggs
203	sterile	fiesta	no_eggs
204	organic	fiesta	eggs
205	sterile	belstar	no_eggs
206	conventional	belstar	no_eggs
207	conventional	fiesta	no_eggs
208	sterile	belstar	eggs
209	organic	fiesta	no_eggs
210	conventional	fiesta	eggs
211	sterile	fiesta	eggs
212	organic	belstar	no_eggs

301	sterile	belstar	eggs
302	sterile	fiesta	eggs
303	organic	fiesta	eggs
304	organic	belstar	eggs
305	conventional	fiesta	eggs
306	conventional	belstar	no_eggs
307	organic	fiesta	no_eggs
308	organic	belstar	no_eggs
309	sterile	fiesta	no_eggs
310	sterile	belstar	no_eggs
311	conventional	belstar	eggs
312	conventional	fiesta	no_eggs

401	organic	belstar	eggs
402	organic	fiesta	no_eggs
403	organic	belstar	no_eggs
404	sterile	fiesta	no_eggs
405	onventiona	belstar	no_eggs
406	sterile	belstar	no_eggs
407	sterile	belstar	eggs
408	onventiona	belstar	eggs
409	organic	fiesta	eggs
410	sterile	fiesta	eggs
411	onventiona	fiesta	eggs
412	onventiona	fiesta	no_eggs

501	conventional	fiesta	eggs
502	sterile	fiesta	eggs
503	organic	fiesta	no_eggs
504	conventional	belstar	no_eggs
505	sterile	belstar	eggs
506	organic	belstar	no_eggs
507	sterile	belstar	no_eggs
508	organic	belstar	eggs
509	conventional	belstar	eggs
510	organic	fiesta	eggs
511	sterile	fiesta	no_eggs
512	conventional	fiesta	no_eggs

601	conventional	belstar	eggs
602	sterile	fiesta	eggs
603	sterile	belstar	no_eggs
604	sterile	belstar	eggs
605	organic	fiesta	no_eggs
606	conventional	fiesta	eggs
607	organic	belstar	eggs
608	sterile	fiesta	no_eggs
609	organic	belstar	no_eggs
610	organic	fiesta	eggs
611	conventional	belstar	no_eggs
612	conventional	fiesta	no_eggs

701	sterile	belstar	eggs
702	conventional	belstar	eggs
703	organic	fiesta	eggs
704	organic	belstar	no_eggs
705	conventional	fiesta	no_eggs
706	sterile	fiesta	no_eggs
707	sterile	fiesta	eggs
708	organic	fiesta	no_eggs
709	conventional	belstar	no_eggs
710	sterile	belstar	no_eggs
711	organic	belstar	eggs
712	conventional	fiesta	eggs

801	sterile	belstar	eggs
802	onventiona	belstar	no_eggs
803	onventiona	belstar	eggs
804	organic	belstar	no_eggs
805	sterile	belstar	no_eggs
806	sterile	fiesta	no_eggs
807	onventiona	fiesta	no_eggs
808	onventiona	fiesta	eggs
809	sterile	fiesta	eggs
810	organic	fiesta	eggs
811	organic	belstar	eggs
812	organic	fiesta	no_eggs

901	sterile	fiesta	eggs
902	conventional	belstar	no_eggs
903	sterile	fiesta	no_eggs
904	sterile	belstar	no_eggs
905	conventional	fiesta	no_eggs
906	sterile	belstar	eggs
907	conventional	fiesta	eggs
908	conventional	belstar	eggs
909	organic	belstar	no_eggs
910	organic	belstar	eggs
911	organic	fiesta	eggs
912	organic	fiesta	no_eggs

1001	conventional	fiesta	eggs
1002	organic	fiesta	no_eggs
1003	sterile	fiesta	no_eggs
1004	organic	belstar	no_eggs
1005	conventional	fiesta	no_eggs
1006	organic	belstar	eggs
1007	sterile	fiesta	eggs
1008	sterile	belstar	eggs
1009	sterile	belstar	no_eggs
1010	conventional	belstar	eggs
1011	conventional	belstar	no_eggs
1012	organic	fiesta	eggs

organic	belstar	eggs	square green
organic	fiesta	eggs	square pink
conventiona	belstar	eggs	square purple
conventiona	fiesta	eggs	square yellow
sterile	belstar	eggs	square blue
sterile	fiesta	eggs	square beige
organic	belstar	no_eggs	square grey
organic	fiesta	no_eggs	square red
conventiona	belstar	no_eggs	round pink
conventiona	fiesta	no_eggs	round yellow
sterile	belstar	no_eggs	round orange
sterile	fiesta	no_eggs	round white

Kinsealy 2015 2nd generation

Annex 12 – Cabbage root fly culture

Material needed

- Mesh cages
- Builders sand
- 25cm pots and saucers
- Organic swedes
- Petri dishes or plastic dishes
- Absorbent cotton wool
- Yeast extract/Marmite
- Granular sugar
- Honey
- Brewer's yeast
- Soya flour
- Masking tape
- Spray bottle with water

10% sucrose solution – KEEP IN THE FRIDGE (or it goes alcoholic and kills flies)
Dissolve 200g of granulated in 2L bottle in room temperature tap water, and shake vigorously.

Fly feed – make as required, do not store premixed
In a petri dish, mix half a tea spoon of honey with half a teaspoon of yeast extract. Smear mixture in several petri dishes (as many as required, 2 per cages is good). Sprinkle brewer's yeast and some soya flour on top of each mixture dish. Take a piece of masking tape, cut it in half lengthwise and put it in the dish, forming a cross directly on top of the mixture, to provide landing platform for flies, and avoid having them drowning in sticky mixture. Make sure no masking tape is curling up, otherwise, flies will stick to it.

Cages care
Cages are kept clean in regularly sweeping dead adults with brush and pan. At the end of adult stage, the empty cage is sprayed with disinfectant and cleaned out. Every couple of generations, the mesh of the cage is removed from the metal structure and wash with washing powder in lukewarm water to remove flies excrements and traces of feed.

Insectary
The insectary is maintained at 16-22°C, and 60±5% relative humidity, with an 18 hour photoperiod.It is regularly deep cleaned to minimize risks of contamination of fungus gnats and Mucor fungi.

Start from pupae (received from Rennes University, UMR IGEPP)
Pupae received are left to emerge in several cages, placed in dishes with 0.5 cm of moist sand.

Adults are provided with:
- A dish of cotton wool soaked in tap water
- A dish of cotton wool soaked in 10% sucrose solution
- One or two dishes of feed mixture with masking tape
- Either cubes of swedes in damp sand (if eggs are extracted) or a whole swede in damp sand in pot, with the top third exposed, as egg laying site.

Feed dishes are refreshed as required (minimum every week) and sucrose solution dishes are changed every three days (every two days if they're drying quickly), as the solution goes alcoholic quite quickly (because of yeast deposition from flies feet). The water dishes are topped up with fresh water every couple of days and cotton wool is changed when required (when there is mould or too many dead adults).

Depending on workload, eggs are either extracted from egg laying sites by simple floatation and inoculated onto fresh organic swedes at 1 egg for 6 g of swede ratio, or fresh organic swedes are provided to flies as egg laying sites, and swedes are removed from the cage every 3 to 5 days (depending on egg laying activity) and placed to incubate in an empty cage. (DO NOT COVER INOCULATED SWEDE WITH CLING FILM, it only accelerate the Mucor growth). If extracting eggs by flotation, greatest care is taken to not damage the eggs, and manipulations are kept to a minimum, with paintbrushes only. Once deposited on the sand next to the swedes, some sand is sprinkled over the eggs to cover them up and avoid desiccation.

Inoculated swedes are sprayed with water every couple of days to avoid egg desiccation.
Once hatched, the larvae don't require any additional moisture as they get enough from the swede itself.

Inoculated swedes are inspected regularly for contamination and any green shouts are removed.

Three weeks after inoculation, the swedes and surrounding sand are inspected for pupae. If pupae are present, the rotten swede is discarded and pupae are extracted from the sand by simple floatation. They are then placed in an empty cage in a dish of moist sand to leave and mature, until hatching.

Annex 13 – Paired farm survey blank information sheet

Farm:

Contact:

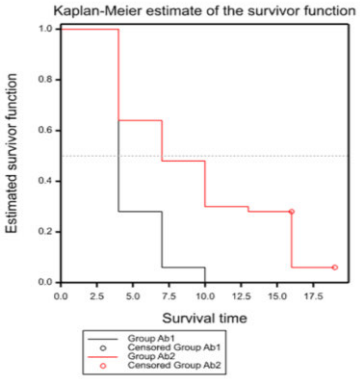
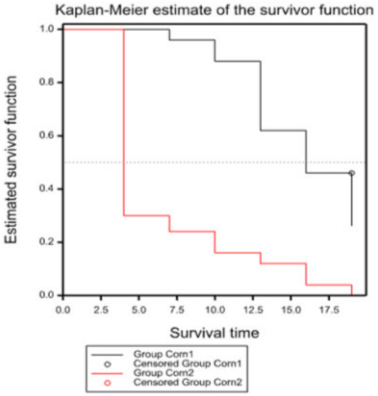
Field information to record

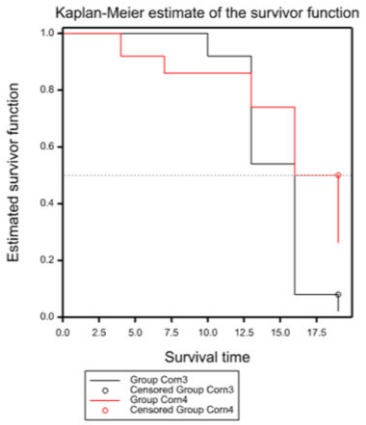
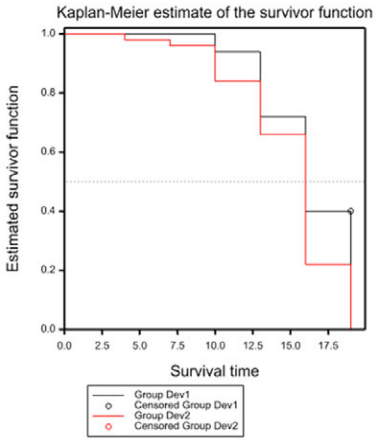
GPS coordinates , postcode, field name or identifier	
Years of organic/conventional management	
Soil fertility input (last 3 years max)	
Crop protection input (last 3 years max)	
Crop rotation type (last 3 years max)	
Type of farm – intensive, high nature value, mixed, small scale…	
Level and timing of cabbage root fly damage (if any)	
Any other recurring pest issue (diamondback moth? Flea beetles? Cabbage whites?)	
Type of brassica grown (swedes, broccoli, OSR…) and yield (could use category high, medium, low)	
Main consumers (veg box, wholesalers, farmers market, packhouses…)	
Can you see yourself being impacted by Brexit? (foreign labour issue, cost of imports, pound value…)	
Anything else that you might want to add about the sampled field or brassica crop this year?	

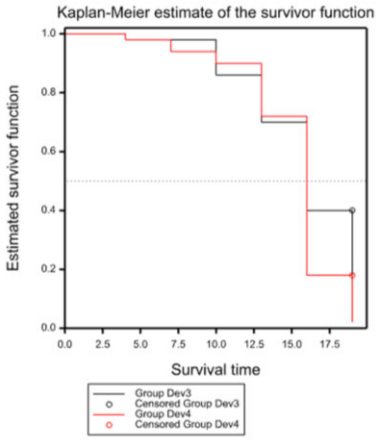
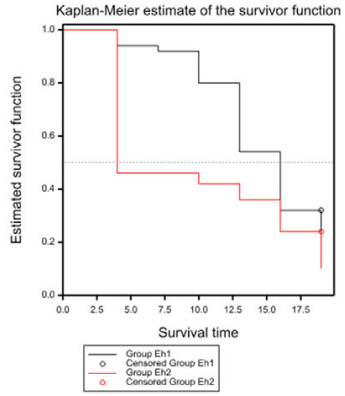
Annex 14 – Paired soil survey baiting survival analysis (Kaplan Meier estimates and t-tests)

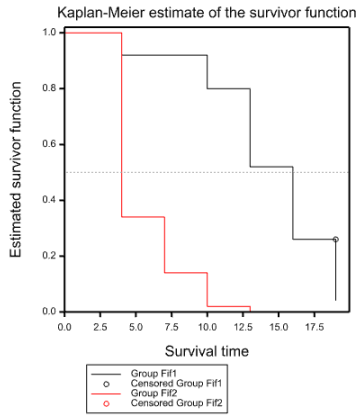
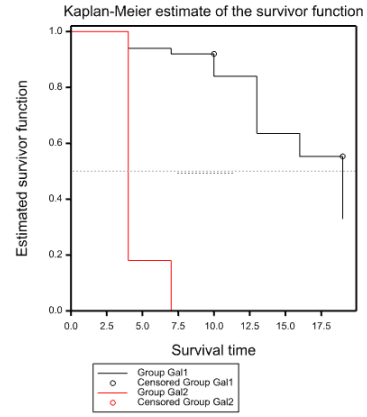
grey cells: NS;
colour coding as followed:

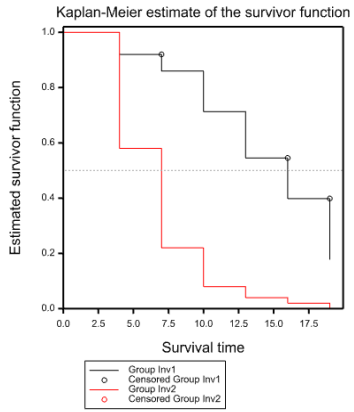
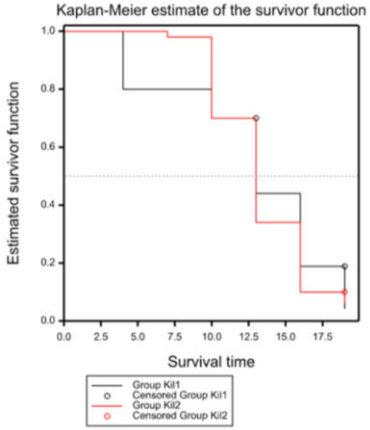
Outcomes	Time points	Soil pairs	Total
No difference in mortality	Kil1+2, Lanc, Linc3+4, North		4/18 pairs
Higher mortality in organic soil	both at 10 and 19 days	Corn1+2, Gal, Inv, Kil3+4, Wex	10/18 pairs
	at 10 days	Eh, Fif, Nber	
	at 19 days	Dev1+2, Dev3+4	
Higher mortality in conventional soil	both at 10 and 19 days	none	4/18 pairs
	at 10 days	Ab, Linc1+2	
	at 19 days	Shef, Corn3+4	
Switch in pattern between 10 and 19 days	none		0/18 pairs

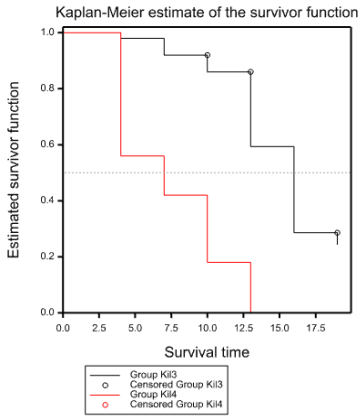
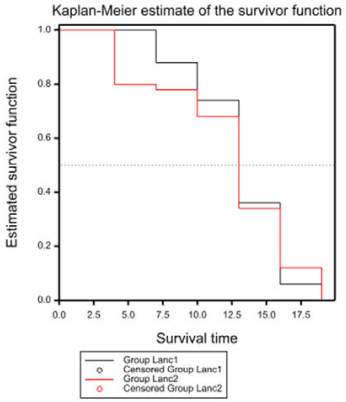
	KM estimate	Mortality at 10D		Mortality at 19D			Notes
		paired t test x1-x2=0	2 sample t test x1<x2	paired t test x1-x2=0	2 sample t test x1<x2		
Ab		Ab1-Ab2=0, t=6.71, df=4, p=0.003	Ab1 NOT < Ab2, t=--6.71, df=8, p=1 *EV error	Ab1-Ab2=0, t=1, df=4, p=0.374	Ab1 NOT < Ab2, t=-1, df=8, p=0.827 *EV error	Mortality of conventional soil higher than organic at mid way point, but organic mortality catch up at the end of the experiment	
Corn1&2		Corn1-Corn2=0, t=-8.37 df=4, p=0.001	Corn1 NOT < Corn2, t=10.18, df=8, <0.001	Corn1-Corn2=0, t=-5.10 df=4, p=0.007	Corn1 NOT < Corn2, t=5.10, df=8, <0.001 *EV error	Mortality of organic soil higher along entire experiment	very high mortality within 3 days most likely due to nematodes

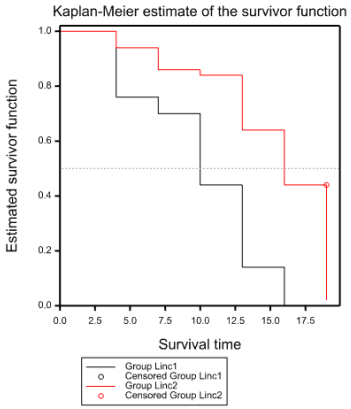
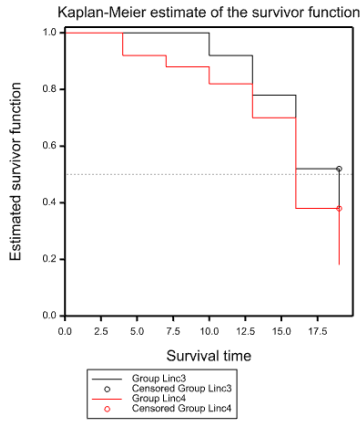
Corn3&4		Corn3-Corn4=0, t=-1.37 df=4, p=0.242	Corn3 NOT < Corn4, t=0.75, df=8, p=0.238	Corn3-Corn4=0, t=2.75 df=4, p=0.0.52	Corn3 NOT < Corn4, t=-2.75, df=8, p=0.987 *EV error	Mortality of conventional soil accelerates at the end of experiment, exceeding organic soil mortality.
Dev1&2		Dev1-Dev2=0, t=-2.24, df=4, p=0.089	Dev1 NOT < Dev2, t=1.27, df=8, p=0.120	Dev1-Dev2=0, t=-2.14, df=4, p=0.099	Dev1 NOT < Dev2, t=2.14, df=8, p=0.032 *EV error	Mortality of organic soil accelerates at the end of experiment, exceeding conventional soil mortality.

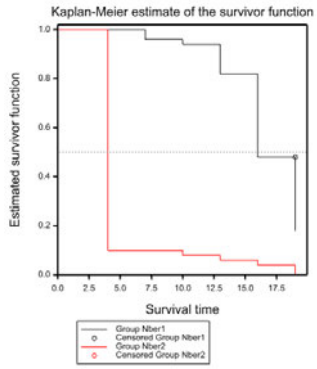
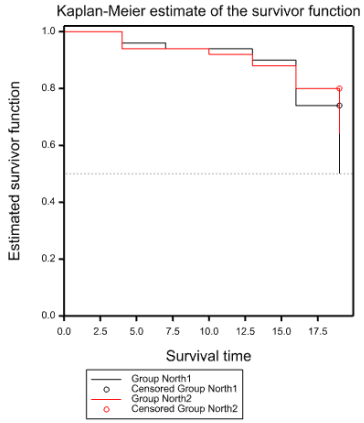
Dev3&4		Dev3-Dev4=0, t=0.78, df=4, p=0.477	Dev3 NOT < Dev4, t=-0.78, df=8, p=0.772	Dev3-Dev4=0, t=-2.24, df=4, p=0.089	Dev3 NOT < Dev4, t=1.89, df=8, p=0.048	Mortality of organic soil accelerates at the end of experiment, exceeding conventional soil mortality.	
Eh		Eh1-Eh2=0, t=-7.74, df=4, p=0.001	Eh1 NOT < Eh2, t=7.76, df=8, p<0.001	Eh1-Eh2=0, t=0, df=4, p=0.5	Eh1 NOT < Eh2, t=0, df=8, p=0.5	Organic soil mortality accelerates before the mid-way point of the experiment, but is later caught up with conventional soil mortality.	very high mortality within 3 days most likely due to nematodes

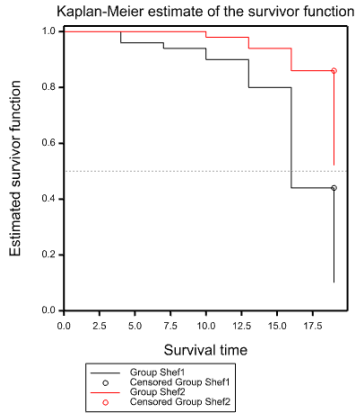
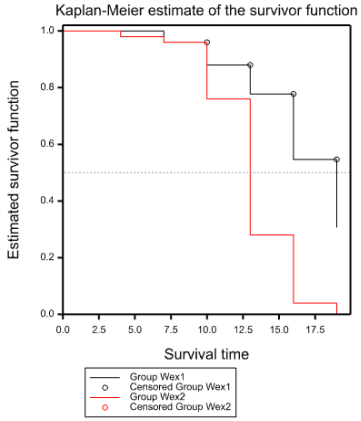
Fif		Fif1-Fif2=0, t=-20.85, df=4, p<0.001	Fif1 NOT < Fif2, t=20.85, df=8, p<0.001	Fif1-Fif2=0, t=-1.63, df=4, p=0.178	Fif1 NOT < Fif2, t=-1.63, df=8, p=0.929 *EV error	Organic soil mortality accelerates before the mid-way point of the experiment, but is later caught up with conventional soil mortality.	very high mortality within 3 days most likely due to nematodes
Gal		Gal1-Gal2=0, t=-35.11, df=4, p<0.001	Gal1 NOT < Gal2, t=35.11, df=8, p<0.001	Gal1-Gal2=0, t=-5.55, df=4, p=0.005	Gal1 NOT < Gal2, t=5.55, df=8, p<0.001 *EV error	Mortality of organic soil higher along entire experiment	very high mortality within 3 days most likely due to nematodes

Inv		Inv1-Inv2=0, t=-9.44, df=4, p<0.001	Inv1 NOT < Inv2, t=6.16, df=8, p<0.001	Inv1-Inv2=0, t=-2.05, df=4, p=0.11	Inv1 NOT < Inv2, t=2.05, df=8, p=0.037, *EV error	Mortality of organic soil higher along entire experiment	
Kil1&2		Kil1-Kil2=0, t=0, df=4, p=1	Kil1 NOT < Kil2, t=0, df=8, p=0.5 *EV error	Kil1-Kil2=0, t=0.78, df=4, p=0.477	Kil1 NOT < Kil2, t=-0.89, df=8, p=0.801	There is no difference in soil mortalities	

Kil3&4		Kil3-Kil4=0, t=-13.88, df=4, p<0.001	Kil3 NOT < Kil4, t=15.21, df=8, p<0.001	Kil3-Kil4=0, t=-3.14, df=4, p=0.035	Kil3 NOT < Kil4, t=3.15, df=8, p=0.007, *EV error	Mortality of organic soil higher along entire experiment	
Lanc		Lanc1-Lanc2=0, t=0, df=4, p=1	Lanc1 NOT < Lanc2, t=0, df=8, p=0.5 *EV error	all dead at 19D		There is no difference in soil mortalities	

Linc1&2		Linc1-Linc2=0, t=3.81, df=4, p=0.019	Linc1 NOT < Linc2, t=-4.42, df=8, p=0.999	Linc1-Linc2=0, t=1, df=4, p=0.187	Linc1 NOT < Linc2, t=1, df=8, p=0.173 * EV error	Mortality of conventional soil higher than organic at mid way point, but organic mortality catch up at the end of the experiment	
Linc3&4		Linc3-Linc4=0, t=-0.97, df=4, p=0.388	Linc3 NOT < Linc4, t=1.04, df=8, p=0.165	Linc3-Linc4=0, t=-0.82, df=4, p=0.46	Linc3 NOT < Linc4, t=1.04, df=8, p=0.164	There is no difference in soil mortalities	

Nber		Nber1-Nber2=0, t=-16.87, df=4, p<0.001	Nber1 NOT < Nber2, t=19.23, df=8, p<0.001	Nber1-Nber2=0, t=1.74, df=4, p=0.08	Nber1 NOT < Nber2, t=1.81, df=8, p=0.69	Organic soil mortality accelerates before the mid-way point of the experiment, but is later caught up with conventional soil mortality.	very high mortality within 3 days most likely due to nematodes
North		North1-North2=0, t=-0.59, df=4, p=0.587	North1 NOT < North2, t=0.59, df=8, p=0.286	North1-North2=0, t=-0.75, df=4, p=0.753	North1 NOT < North2, t=-0.94, df=8, p=0.812	There is no difference in soil mortalities	very low overall mortality

Shef		Shef1-Shef2=0, t=2.14, df=4, p=0.099	Shef1 NOT < Shef2, t=-2.14, df=8, p=0.968	Shef1-Shef2=0, t=4.78, df=4, p=0.009	Shef1 NOT < Shef2, t=-4.85, df=8, p=0.999	Mortality of conventional soil accelerates at the end of experiment, exceeding organic soil mortality.	
Wex		Wex1-Wex2=0, t=-1.63, df=4, p=0.178	Wex1 NOT < Wex2, t=1.90, df=8, p=0.047	Wex1-Wex2=0, t=-3.58, df=4, p=0.023	Wex1 NOT < Wex2, t=3.58, df=8, p=0.004	Mortality of organic soil higher along entire experiment	

References

Abdollahzadeh, G., Damalas, C.A., Sharifzadeh, M.S., 2017. Understanding adoption, non-adoption, and discontinuance of biological control in rice fields of northern Iran. Crop Prot. 93, 60–68. https://doi.org/10.1016/j.cropro.2016.11.014

AHDB, 2019. Pest insects infesting Brassica crops 1–10.

AHDB, 2015. Brassicas : module drenches to control cabbage root fly - project FV 416a.

AHDB, 2013. Brassicas: module drenches to control cabbage root fly - project FV 416.

Albizua, A., Williams, A., Hedlund, K., Pascual, U., 2015. Crop rotations including ley and manure can promote ecosystem services in conventional farming systems. Appl. Soil Ecol. 95, 54–61. https://doi.org/10.1016/j.apsoil.2015.06.003

Alborn, H., Karlsson, H., Lundgren, L., Ruuth, P., Stenhagen, G., 1985. Resistance in crop species of the genus Brassica to oviposition by the turnip root fly , Hylemya floralis. Oikos 44, 61–69.

Altieri, M. A, Nicholls, C.I., 2012. Sustainable Agriculture Reviews, Sustainable Agriculture Reviews. https://doi.org/10.1007/978-94-007-5449-2

Altieri, M.A., 1999. The ecological role of biodiversity in agroecosystems. Agric. Ecosyst. Environ. 74, 19–31. https://doi.org/10.1016/S0167-8809(99)00028-6

Altieri, M.A., 1995. Agroecology: The Science Of Sustainable Agriculture. Westview Press.

Altieri, M.A., Nicholls, C.I., 2020. Agroecology and the reconstruction of a post-COVID-19 agriculture. Https://Doi.Org/10.1080/03066150.2020.1782891 0, 1–18. https://doi.org/10.1080/03066150.2020.1782891

Altieri, M.A., Nicholls, C.I., 2005. Agroecology and the Search for a Truly Sustainable Agriculture. PNUMA United Nations Environment Programme.

Altieri, M.A., Nicholls, C.I., 2004a. Biodiversity and Pest Management in Agroecosystems, 2nd editio. ed. Food Products Press.

Altieri, M.A., Nicholls, C.I., 2004b. An agroecological basis for designing diversified cropping systems in the tropics. J. Crop Improv. 11, 81–103. https://doi.org/10.1300/J411v11n01_05

Altieri, M.A., Nicholls, C.I., 2003. Soil fertility management and insect pests: harmonizing soil and plant health in agroecosystems. Soil Tillage Res. 72, 203–211. https://doi.org/10.1016/S0167-1987(03)00089-8

Altieri, M.A., Nicholls, C.I., Henao, A., Lana, M.A., 2015. Agroecology and the design of climate change-resilient farming systems. Agron. Sustain. Dev. 35, 869–890. https://doi.org/10.1007/s13593-015-0285-2

Altieri, M. A, Nicholls, C.I.C.I., Fritz, M.A., 2005a. Manage Insects on Your Farm: A Guide to

Ecological Strategies. Sustainable Agriculture Network.

Altieri, M.A, Ponti, L., Nicholls, C.I., 2005b. Enhanced Pest Management Through Soil Health : Toward a Belowground Habitat Management Strategy. Biodynamics.

Altieri, M.A., Schmidt, L.L., Montalba, R., 1998. Assessing the effects of agroecological soil management practices on broccoli insect pest populations. Bio-dynamics 23–26.

Altieri, M.A., Toledo, V.M., 2011. The agroecological revolution in Latin America: Rescuing nature, ensuring food sovereignty and empowering peasants. J. Peasant Stud. 38, 587–612. https://doi.org/10.1080/03066150.2011.582947

Alyokhin, A., Nault, B., Brown, B., 2019. Soil conservation practices for insect pest management in highly disturbed agroecosystems – a review. Entomol. Exp. Appl. 7–27. https://doi.org/10.1111/eea.12863

Alyokhin, A., Porter, G., Groden, E., Drummond, F., 2005. Colorado potato beetle response to soil amendments: A case in support of the mineral balance hypothesis? Agric. Ecosyst. Environ. 109, 234–244. https://doi.org/10.1016/j.agee.2005.03.005

Amundson, R., Berhe, A.A., Hopmans, J.W., Olson, C., Sztein, A.E., Sparks, D.L., 2015. Soil and human security in the 21st century. Science (80-.). 348, 1261071–1261071. https://doi.org/10.1126/science.1261071

Andersen, A., Hansen, Å.G., Rydland, N., ØYre, G., 1983. Carabidae and Staphylinidae (Col.) as predators of eggs of the turnip root fly Delia floralis Fallén (Diptera, Anthomyiidae) in cage experiments. Zeitschrift für Angew. Entomol. 95, 499–506. https://doi.org/10.1111/j.1439-0418.1983.tb02673.x

Ansari, M.A., Tirry, L., Moens, M., 2005. Antagonism between entomopathogenic fungi and bacterial symbionts of entomopathogenic nematodes. BioControl 50, 465–475. https://doi.org/10.1007/s10526-004-5524-4

Armstrong Brown, S.M., Cook, H.F., Lee, H.C., 2000. Topsoil Characteristics from a Paired Farm Survey of Organic versus Conventional Farming in Southern England. Biol. Agric. Hortic. 18, 37–54. https://doi.org/10.1080/01448765.2000.9754863

Asteraki, E.J., Hanks, C.B., Clements, R.O., 1992. The impact of two insecticides on predatory ground beetles (Carabidae) in newly-sown grass. Ann. Appl. Biol. 120, 25–39. https://doi.org/10.1111/j.1744-7348.1992.tb03400.x

Athey, K.J., Dreyer, J., Kowles, K.A., Penn, H.J., Sitvarin, M.I., Harwood, J.D., 2016. Spring Forward: molecular detection of early season predation in agroecosystems. Food Webs 9, 25–31. https://doi.org/10.1016/j.fooweb.2016.06.001

Bale, J. S., van Lenteren, J.. C., Bigler, F., 2008. Biological control and sustainable food production. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 363, 761–76. https://doi.org/10.1098/rstb.2007.2182

Barbercheck, M.E., Kaya, H.K., 1991. Competitive interactions between entomopathogenic nematodes and Beauveria bassiana (Deuteromycotina: Hyphomycetes) in soilborne

larvae of Spodoptera exigua (Lepidoptera: Noctuidae). Environ. Entomol. 20, 707–712. https://doi.org/10.1093/ee/20.2.707

Bardgett, R.D., Van Der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. Nature 515, 505–511. https://doi.org/10.1038/nature13855

Barelli, L., Moonjely, S., Behie, S.W., Bidochka, M.J., 2016. Fungi with multifunctional lifestyles: endophytic insect pathogenic fungi. Plant Mol. Biol. 90, 657–664. https://doi.org/10.1007/s11103-015-0413-z

Barron, M.G., Woodburn, K.B., 1995. Ecotoxicology of chlorpyrifos. Rev. Environ. Contam. Toxicol. https://doi.org/10.1007/978-1-4612-2550-8_1

Bartual, A.M., Sutter, L., Bocci, G., Moonen, A.-C., Cresswell, J., Entling, M., Giffard, B., Jacot, K., Jeanneret, P., Holland, J., Pfister, S., Pintér, O., Veromann, E., Winkler, K., Albrecht, M., 2019. The potential of different semi-natural habitats to sustain pollinators and natural enemies in European agricultural landscapes. Agric. Ecosyst. Environ. 279, 43–52. https://doi.org/10.1016/J.AGEE.2019.04.009

Barzman, M., Bàrberi, P., Birch, A.N.E., Boonekamp, P., Dachbrodt-Saaydeh, S., Graf, B., Hommel, B., Jensen, J.E., Kiss, J., Kudsk, P., Lamichhane, J.R., Messéan, A., Moonen, A.C., Ratnadass, A., Ricci, P., Sarah, J.L., Sattin, M., 2015. Eight principles of integrated pest management. Agron. Sustain. Dev. 35, 1199–1215. https://doi.org/10.1007/s13593-015-0327-9

Barzman, M., Dachbrodt-Saaydeh, S., 2011. Comparative analysis of pesticide action plans in five European countries. Pest Manag. Sci. 67, 1481–1485. https://doi.org/10.1002/ps.2283

Batáry, P., Báldi, A., Kleijn, D., Tscharntke, T., 2011. Landscape-moderated biodiversity effects of agri-environmental management: A meta-analysis. Proc. R. Soc. B Biol. Sci. 278, 1894–1902. https://doi.org/10.1098/rspb.2010.1923

Baur, R., Birch, A. N.E., Hopkins, R.J., Griffiths, D.W., Simmonds, M.S.J., Städler, E., 1996a. Oviposition and chemosensory stimulation of the root flies Delia radicum and D. floralis in response to plants and leaf surface extracts from resistant and susceptible Brassica genotypes. Entomol. Exp. Appl. 78, 61–75. https://doi.org/10.1111/j.1570-7458.1996.tb00765.x

Baur, R., Kosal, V., Patrian, B., Stadler, E., 1996b. Preference for plants damaged by conspecific larvae in ovipositing cabbage root flies: influence of stimuli from leaf surface and roots. Entomol. Exp. Appl. 81, 353–364. https://doi.org/10.1046/j.1570-7458.1996.00106.x

Beck, B., Spanoghe, P., Moens, M., Brusselman, E., Temmerman, F., Pollet, S., Nuyttens, D., 2014. Improving the biocontrol potential of Steinernema feltiae against Delia radicum through dosage, application technique and timing. Pest Manag. Sci. 70, 841–51. https://doi.org/10.1002/ps.3628

Begg, G.S., Cook, S.M., Dye, R., Ferrante, M., Franck, P., Lavigne, C., Lövei, G.L., Mansion-Vaquie, A., Pell, J.K., Petit, S., Quesada, N., Ricci, B., Wratten, S.D., Birch, A.N.E., 2017.

A functional overview of conservation biological control. Crop Prot. 97, 145–158. https://doi.org/10.1016/j.cropro.2016.11.008

Behie, S.W., Zelisko, P.M., Bidochka, M.J., 2012. Endophytic Insect-Parasitic Fungi 14256, 1576–1578.

Bender, S.F., Wagg, C., van der Heijden, M.G.A., 2016. An Underground Revolution: Biodiversity and Soil Ecological Engineering for Agricultural Sustainability. Trends Ecol. Evol. 31, 440–452. https://doi.org/10.1016/j.tree.2016.02.016

Bengtsson, J., Ahnstrom, J., Weibull, A.C., 2005. The effects of organic agriculture on biodiversity and abundance: A meta-analysis. J. Appl. Ecol. 42, 261–269. https://doi.org/10.1111/j.1365-2664.2005.01005.x

Bhogal, A., Nicholson, F.A., Chambers, B.J., 2009. Organic carbon additions: Effects on soil bio-physical and physico-chemical properties. Eur. J. Soil Sci. 60, 276–286. https://doi.org/10.1111/j.1365-2389.2008.01105.x

Bianchi, F., Booij, C., Tscharntke, T., 2006. Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. Proc. Biol. Sci. 273, 1715–1727. https://doi.org/10.1098/rspb.2006.3530

Bing, L.A., Lewis, L.C., 1993. Occurrence of the entomopathogen Beauveria bassiana (Balsamo) Vuillemin in different tillage regimes and in Zea mays L. and virulence towards Ostrinia nubilalis (Hübner). Agric. Ecosyst. Environ. 45, 147–156. https://doi.org/10.1016/0167-8809(93)90065-W

Birkhofer, K., Bezemer, T.M., Bloem, J., Bonkowski, M., Christensen, S., Dubois, D., Ekelund, F., Fließbach, A., Gunst, L., Hedlund, K., Mäder, P., Mikola, J., Robin, C., Setälä, H., Tatin-Froux, F., Van der Putten, W.H., Scheu, S., 2008a. Long-term organic farming fosters below and aboveground biota: Implications for soil quality, biological control and productivity. Soil Biol. Biochem. 40, 2297–2308. https://doi.org/10.1016/j.soilbio.2008.05.007

Birkhofer, K., Bylund, H., Dalin, P., Ferlian, O., Gagic, V., Hambäck, P.A., Klapwijk, M., Mestre, L., Roubinet, E., Schroeder, M., Stenberg, J.A., Porcel, M., Björkman, C., Jonsson, M., 2017. Methods to identify the prey of invertebrate predators in terrestrial field studies. Ecol. Evol. https://doi.org/10.1002/ece3.2791

Birkhofer, K., Wise, D.H., Scheu, S., 2008b. Subsidy from the detrital food web, but not microhabitat complexity, affects the role of generalist predators in an aboveground herbivore food web. Oikos 117, 494–500. https://doi.org/10.1111/j.0030-1299.2008.16361.x

Björkman, M., 2007. Effects of Intercropping on the Life Cycle of the Turnip Root Fly (Delia floralis).

Björkman, M., Hambäck, P. a., Hopkins, R.J., Rämert, B., 2010. Evaluating the enemies hypothesis in a clover-cabbage intercrop: effects of generalist and specialist natural enemies on the turnip root fly (Delia floralis). Agric. For. Entomol. 12, 123–132. https://doi.org/10.1111/j.1461-9563.2009.00452.x

Björkman, M., Hambäck, P.A., Rämert, B., 2007. Neighbouring monocultures enhance the effect of intercropping on the turnip root fly (Delia floralis). Entomol. Exp. Appl. 124, 319–326. https://doi.org/10.1111/j.1570-7458.2007.00589.x

Blouin, M., Zuily-Fodil, Y., Pham-Thi, A.-T., Laffray, D., Reversat, G., Pando, A., Tondoh, J., Lavelle, P., 2005. Belowground organism activities affect plant aboveground phenotype, inducing plant tolerance to parasites. Ecol. Lett. 8, 202–208. https://doi.org/10.1111/j.1461-0248.2004.00711.x

Bommarco, R., Kleijn, D., Potts, S.G., 2013. Ecological intensification: Harnessing ecosystem services for food security. Trends Ecol. Evol. 28, 230–238. https://doi.org/10.1016/j.tree.2012.10.012

Borrelli, P., Robinson, D.A., Fleischer, L.R., Lugato, E., Ballabio, C., Alewell, C., Meusburger, K., Modugno, S., Schütt, B., Ferro, V., Bagarello, V., Oost, K. Van, Montanarella, L., Panagos, P., 2017. An assessment of the global impact of 21st century land use change on soil erosion. Nat. Commun. 8. https://doi.org/10.1038/s41467-017-02142-7

Bretagnolle, V., Berthet, E., Gross, N., Gauffre, B., Plumejeaud, C., Houte, S., Badenhausser, I., Monceau, K., Allier, F., Monestiez, P., Gaba, S., 2018. Towards sustainable and multifunctional agriculture in farmland landscapes: Lessons from the integrative approach of a French LTSER platform. Sci. Total Environ. 627, 822–834. https://doi.org/10.1016/j.scitotenv.2018.01.142

Brévault, T., Clouvel, P., 2019. Pest management: Reconciling farming practices and natural regulations. Crop Prot. 115, 1–6. https://doi.org/10.1016/j.cropro.2018.09.003

Brewer, M.J., Goodell, P.B., 2012. Approaches and Incentives to Implement Integrated Pest Management that Addresses Regional and Environmental Issues. Annu. Rev. Entomol. 57, 41–59. https://doi.org/10.1146/annurev-ento-120709-144748

Brittan, G., Bandyopadhyay, P.S., 2019. Ecology, Evidence, and Objectivity: In Search of a Bias-Free Methodology. Front. Ecol. Evol. 7, 399. https://doi.org/10.3389/fevo.2019.00399

Broatch, J.S., Dosdall, L.M., Clayton, G.W., Harker, K.N., Yang, R.-C., 2006. Using Degree-Day and Logistic Models to Predict Emergence Patterns and Seasonal Flights of the Cabbage Maggot and Seed Corn Maggot (Diptera: Anthomyiidae) in Canola. Environ. Entomol. 35, 1166–1177. https://doi.org/10.1093/ee/35.5.1166

Broatch, J.S., Dosdall, L.M., O'Donovan, J.T., Harker, K.N., Clayton, G.W., 2010. Responses of the specialist biological control agent, Aleochara bilineata, to vegetational diversity in canola agroecosystems. Biol. Control 52, 58–67. https://doi.org/10.1016/j.biocontrol.2009.08.009

Bruck, D.J., Snelling, J.E., Dreves, A.J., Jaronski, S.T., 2005. Laboratory bioassays of entomopathogenic fungi for control of Delia radicum (L.) larvae. J. Invertebr. Pathol. 89, 179–83. https://doi.org/10.1016/j.jip.2005.02.007

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., Peplies, J., Gloeckner, F.O.,

Amann, R., Eickhorst, T., Schulze-Lefert, P., 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature 488, 91–5. https://doi.org/10.1038/nature11336

Butler, D., 2018. EU pesticide review could lead to ban: Major assessment concludes that neonicotinoids harm bees. Nature. https://doi.org/10.1038/d41586-018-02639-1

Campbell, J.F., Gaugler, R.R., 2010. Nictation Behaviour and Its Ecological Implications in the Host Search Strategies of Entomopathogenic Nematodes (Heterorhabditidae and Steinernematidae). Behaviour 126, 155–169.

Campos-Herrera, R., Ali, J.G., Diaz, B.M., Duncan, L.W., 2013. Analyzing spatial patterns linked to the ecology of herbivores and their natural enemies in the soil. Front. Plant Sci. 4, 378. https://doi.org/10.3389/fpls.2013.00378

Campos-Herrera, R., El-Borai, F.E., Duncan, L.W., 2015. Modifying soil to enhance biological control of belowground dwelling insects in citrus groves under organic agriculture in Florida. Biol. Control 84, 53–63. https://doi.org/10.1016/j.biocontrol.2015.02.002

Campos-Herrera, R., El-Borai, F.E., Duncan, L.W., 2012. Wide interguild relationships among entomopathogenic and free-living nematodes in soil as measured by real time qPCR. J. Invertebr. Pathol. 111, 126–135. https://doi.org/10.1016/j.jip.2012.07.006

Campos-Herrera, R., Gómez-Ros, J.M., Escuer, M., Cuadra, L., Barrios, L., Gutiérrez, C., 2008. Diversity, occurrence, and life characteristics of natural entomopathogenic nematode populations from La Rioja (Northern Spain) under different agricultural management and their relationships with soil factors. Soil Biol. Biochem. 40, 1474–1484. https://doi.org/10.1016/j.soilbio.2008.01.002

Campos-Herrera, R., Piedra-Buena, A., Escuer, M., Montalbán, B., Gutiérrez, C., 2010. Effect of seasonality and agricultural practices on occurrence of entomopathogenic nematodes and soil characteristics in La Rioja (Northern Spain). Pedobiologia (Jena). 53, 253–258. https://doi.org/10.1016/j.pedobi.2009.11.004

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., MacE, G.M., Tilman, D., Wardle, D.A., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B., Larigauderie, A., Srivastava, D.S., Naeem, S., 2012. Biodiversity loss and its impact on humanity. Nature 486, 59–67. https://doi.org/10.1038/nature11148

Chabert, A., Sarthou, J.-P., 2017. Practices of conservation agriculture prevail over cropping systems and landscape heterogeneity in understanding the ecosystem service of aphid biocontrol. Agric. Ecosyst. Environ. 249, 70–79. https://doi.org/10.1016/j.agee.2017.08.005

Chabert, A., Sarthou, J.P., 2020. Conservation agriculture as a promising trade-off between conventional and organic agriculture in bundling ecosystem services. Agric. Ecosyst. Environ. 292, 106815. https://doi.org/10.1016/j.agee.2019.106815

Chandler, D., Davidson, G., 2005. Evaluation of entomopathogenic fungus Metarhizium anisopliae against soil-dwelling stages of cabbage maggot (Diptera: Anthomyiidae) in glasshouse and field experiments and effect of fungicides on fungal activity. J. Econ.

Entomol. 98, 1856–62.

Chandler, D., Hay, D., Reid, A.P., 1997. Sampling and occurrence of entomopathogenic fungi and nematodes in UK soils. Appl. Soil Ecol. 5, 133–141. https://doi.org/10.1016/S0929-1393(96)00144-8

Chaplin-Kramer, R., O'Rourke, M., Schellhorn, N., Zhang, W., Robinson, B.E., Gratton, C., Rosenheim, J.A., Tscharntke, T., Karp, D.S., 2019. Measuring What Matters: Actionable Information for Conservation Biocontrol in Multifunctional Landscapes. Front. Sustain. Food Syst. 3, 1–10. https://doi.org/10.3389/fsufs.2019.00060

Chaplin-Kramer, R., O'Rourke, M.E., Blitzer, E.J., Kremen, C., 2011. A meta-analysis of crop pest and natural enemy response to landscape complexity. Ecol. Lett. 14, 922–932. https://doi.org/10.1111/j.1461-0248.2011.01642.x

Charles, H., Godfray, H., Garnett, T., 2014. Food security and sustainable intensification. Philos. Trans. R. Soc. B Biol. Sci. 369, 6–11. https://doi.org/10.1098/rstb.2012.0273

Chen, Shulong, Han, X., Moens, M., 2003. Biological control of Delia radicum (Diptera: Anthomyiidae) with entomopathogenic nematodes. Appl. Entomol. Zool. 38, 441–448. https://doi.org/10.1303/aez.2003.441

Chen, S., Li, J., Han, X., Moens, M., 2003. Effect of temperature on the pathogenicity of entomopathogenic nematodes (Steinernema and Heterorhabditis spp.) to Delia radicum. BioControl 48, 713–724. https://doi.org/10.1023/A:1026341325264

Clifton, E.H., Jaronski, S.T., Hodgson, E.W., Gassmann, A.J., 2015. Abundance of soil-borne entomopathogenic fungi in organic and conventional fields in the Midwestern USA with an emphasis on the effect of herbicides and fungicides on fungal persistence. PLoS One 10. https://doi.org/10.1371/journal.pone.0133613

Coaker, T., 1969. New approaches to cabbage root fly control, in: Proc. 5th British Insecticide and Fungicide Conf. pp. 704–710.

Collier, R., Mazzi, D., Schjøll, A.F., Schorpp, Q., Thöming, G., Johansen, T.J., Meadow, R., Meyling, N. V, Cortesero, A., Vogler, U., Gaffney, M.T., Hommes, M., Ga, M.T., Hommes, M., 2020. The Potential for Decision Support Tools to Improve the Management of Root-Feeding Fly Pests of Vegetables in Western Europe 1–16. https://doi.org/10.3390/insects11060369

Collier, R.H., Finch, S., 1985. Accumulated temperatures for predicting the time of emergence in the spring of the cabbage root fly, Delia radicum (L.) (Diptera: Anthomyiidae). Bull. Entomol. Res. 75, 395–404. https://doi.org/10.1017/S0007485300014504

Collier, R.H., Finch, S., Phelps, K., Thompson, A.R., 1991. Possible impact of global warming on cabbage root fly (Delia radicum) activity in the UK. Ann. Appl. Biol. 118, 261–271. https://doi.org/10.1111/j.1744-7348.1991.tb05627.x

Cook, S.M., Khan, Z.R., Pickett, J.A., 2007. The Use of Push-Pull Strategies in Integrated Pest Management. Annu. Rev. Entomol. 52, 375–400.

https://doi.org/10.1146/annurev.ento.52.110405.091407

Cooper, J., Sanderson, R., Cakmak, I., Ozturk, L., Shotton, P., Carmichael, A., Haghighi, R.S., Tetard-jones, C., Volakakis, N., Eyre, M., Leifert, C., 2011. Effect of Organic and Conventional Crop Rotation , Fertilization , and Crop Protection Practices on Metal Contents in Wheat (Triticum aestivum). J. Agric. Food Chem.

Crain, P.R., 2016. Finding the economics in economic entomology: The last 8 years, in: Proceedings of the International Congress of Entomology,. pp. 1–7. https://doi.org/10.1603/ice.2016.93311

Crespo, E., Hordijk, C. a, de Graaf, R.M., Samudrala, D., Cristescu, S.M., Harren, F.J.M., van Dam, N.M., 2012. On-line detection of root-induced volatiles in Brassica nigra plants infested with Delia radicum L. root fly larvae. Phytochemistry 84, 68–77. https://doi.org/10.1016/j.phytochem.2012.08.013

Cullen, R., Warner, K.D., Jonsson, M., Wratten, S.D., 2008. Economics and adoption of conservation biological control. Biol. Control 45, 272–280. https://doi.org/10.1016/j.biocontrol.2008.01.016

Culliney, T.W., 2014. Crop losses to arthropods, in: Integrated Pest Management: Pesticide Problems, Vol.3. Springer Netherlands, pp. 201–225. https://doi.org/10.1007/978-94-007-7796-5_8

Cunningham, S.A., Attwood, S.J., Bawa, K.S., Benton, T.G., Broadhurst, L.M., Didham, R.K., McIntyre, S., Perfecto, I., Samways, M.J., Tscharntke, T., Vandermeer, J., Villard, M.A., Young, A.G., Lindenmayer, D.B., 2013. To close the yield-gap while saving biodiversity will require multiple locally relevant strategies. Agric. Ecosyst. Environ. 173, 20–27. https://doi.org/10.1016/j.agee.2013.04.007

Dainese, M., Martin, E.A., Aizen, M.A., Albrecht, M., Bartomeus, I., Bommarco, R., Carvalheiro, L.G., Chaplin-kramer, R., Gagic, V., Garibaldi, L.A., Ghazoul, J., Grab, H., Jonsson, M., Karp, D.S., Letourneau, D.K., Marini, L., Poveda, K., Rader, R., Smith, H.G., Takada, M.B., Taki, H., Tamburini, G., Tschumi, M., 2019. A global synthesis reveals biodiversity-mediated benefits for crop production 1–14.

Dalgaard, T., Hutchings, N.J., Porter, J.R., 2003. Agroecology, scaling and interdisciplinarity. Agric. Ecosyst. Environ. 100, 39–51. https://doi.org/10.1016/S0167-8809(03)00152-X

Darnhofer, I., 2020. Farm resilience in the face of the unexpected: lessons from the COVID-19 pandemic. Agric. Human Values. https://doi.org/10.1007/s10460-020-10053-5

De Ponti, T., Rijk, B., Van Ittersum, M.K., 2012. The crop yield gap between organic and conventional agriculture. Agric. Syst. 108, 1–9. https://doi.org/10.1016/j.agsy.2011.12.004

De Schutter, O., Vanloqueren, G., 2011. The New Green Revolution : How Twenty-First-Century Science Can Feed the World. Solutions 2, 1–12.

de Vries, F.T., Thébault, E., Liiri, M., Birkhofer, K., Tsiafouli, M. a, Bjørnlund, L., Bracht Jørgensen, H., Brady, M.V., Christensen, S., de Ruiter, P.C., d'Hertefeldt, T., Frouz, J.,

Hedlund, K., Hemerik, L., Hol, W.H.G., Hotes, S., Mortimer, S.R., Setälä, H., Sgardelis, S.P., Uteseny, K., van der Putten, W.H., Wolters, V., Bardgett, R.D., 2013. Soil food web properties explain ecosystem services across European land use systems. Proc. Natl. Acad. Sci. U. S. A. 110, 14296–301. https://doi.org/10.1073/pnas.1305198110

Decaëns, T., Jiménez, J.J., Gioia, C., Measey, G.J., Lavelle, P., 2006. The values of soil animals for conservation biology. Eur. J. Soil Biol. 42. https://doi.org/10.1016/j.ejsobi.2006.07.001

DEFRA, 2020a. Horticulture Statistics 2019.

DEFRA, 2020b. Environmental Land Management: Policy discussion document 38.

DEFRA, 2018. Using nitrogen fertilisers in nitrate vulnerable zones - GOV.UK [WWW Document]. URL https://www.gov.uk/guidance/using-nitrogen-fertilisers-in-nitrate-vulnerable-zones#how-much-organic-manure-you-can-use-farm-and-field-limits (accessed 5.6.20).

DEFRA, 2002. Biocontrol of the cabbage root fly by the release of predators.

Dicks, L. V., Wright, H.L., Ashpole, J.E., Hutchison, J., McCormack, C.G., Livoreil, B., Zulka, K.P., Sutherland, W.J., 2016. What works in conservation? Using expert assessment of summarised evidence to identify practices that enhance natural pest control in agriculture. Biodivers. Conserv. 25, 1383–1399. https://doi.org/10.1007/s10531-016-1133-7

Dixon, P.L., Coady, J.R., Larson, D.J., Spaner, D., 2004. Undersowing rutabaga with white clover: impact on Delia radicum (Diptera: Anthomyiidae) and its natural enemies. Can. Entomol. 136, 427–442. https://doi.org/10.4039/n03-067

Domínguez, A., Bedano, J.C., 2016. Earthworm and Enchytraeid Co-occurrence Pattern in Organic and Conventional Farming. Soil Sci. 181, 148–156. https://doi.org/10.1097/SS.0000000000000146

Doré, T., Makowski, D., Malézieux, E., Munier-Jolain, N., Tchamitchian, M., Tittonell, P., 2011. Facing up to the paradigm of ecological intensification in agronomy: Revisiting methods, concepts and knowledge. Eur. J. Agron. 34, 197–210. https://doi.org/10.1016/j.eja.2011.02.006

Dosdall, L.M., Florence, L.Z., Conway, P.M., Cowle, N.T., 1998. Tillage regime, row spacing, and seeding rate influence infestations of root maggots (*Delia* spp.) (Diptera: Anthomyiidae) in canola. Can. J. Plant Sci. 78, 671–681. https://doi.org/10.4141/P98-001

Dosdall, L.M., Good, A., Keddie, B.A., Ekuere, U., Stringam, G., 2000. Identification and evaluation of root maggot (Delia spp.) (Diptera: Anthomyiidae) resistance within Brassicaceae. Crop Prot. 19, 247–253. https://doi.org/10.1016/S0261-2194(00)00015-6

Dosdall, L.M., Harker, K.N., O'Donovan, J.T., Blackshaw, R.E., Kutcher, H.R., Gan, Y., Johnson, E.N., 2012. Crop sequence effects on root maggot (Diptera: Anthomyiidae:

Delia spp.) infestations in canola. J. Econ. Entomol. 105, 1261–7.

Dosdall, L.M., Herbut, M.J., Cowle, N.T., Micklich, T.M., 1996. The effect of seeding date and plant density on infestations of root maggots, Delia spp. (Diptera: Anthomyiidae), in canola. Can. J. Plant Sci. 76, 169–177. https://doi.org/10.4141/cjps96-035

Douwe van der Ploeg, J., Barjolle, D., Bruil, J., Brunori, G., Costa Madureira, L.M., van der Ploeg, J.D., Barjolle, D., Bruil, J., Brunori, G., Costa Madureira, L.M., Dessein, J., Drąg, Z., Fink-Kessler, A., Gasselin, P., Gonzalez de Molina, M., Gorlach, K., Jürgens, K., Kinsella, J., Kirwan, J., Knickel, K., Lucas, V., Marsden, T., Maye, D., Migliorini, P., Milone, P., Noe, E., Nowak, P., Parrott, N., Peeters, A., Rossi, A., Schermer, M., Ventura, F., Visser, M., Wezel, A., 2019. The economic potential of agroecology: Empirical evidence from Europe [WWW Document]. J. Rural Stud. https://doi.org/10.1016/j.jrurstud.2019.09.003

Eigenbrode, S.D., Birch, A.N.E., Lindzey, S., Meadow, R., Snyder, W.E., 2016. A mechanistic framework to improve understanding and applications of push-pull systems in pest management. J. Appl. Ecol. 53, 202–212. https://doi.org/10.1111/1365-2664.12556

Eigenbrode, S.D., Pimentel, D., 1988. Effects of manure and chemical fertilizers on insect pest populations on collards. Agric. Ecosyst. Environ. 20, 109–125. https://doi.org/10.1016/0167-8809(88)90151-X

Eilenberg, J., Damgaard, P.H., Hansen, B.M., Pedersen, J.C., Bresciani, J., Larsson, R., 2000. Natural coprevalence of Strongwellsea castrans, Cystosporogenes deliaradicae, and Bacillus thuringiensis in the host, Delia radicum. J. Invertebr. Pathol. 75, 69–75. https://doi.org/10.1006/jipa.1999.4892

Eisenhauer, N., Sabais, A.C.W., Schonert, F., Scheu, S., 2010. Soil arthropods beneficially rather than detrimentally impact plant performance in experimental grassland systems of different diversity. Soil Biol. Biochem. 42, 1418–1424. https://doi.org/10.1016/j.soilbio.2010.05.001

Ellis, P.R., Pink, D.A.C., Barber, N.E., Mead, A., 1999. Identification of high levels of resistance to cabbage root fly, Delia radicum, in wild Brassica species. Euphytica 110, 207–214. https://doi.org/10.1023/A:1003752801143

Ellison, A.M., 2004. Bayesian inference in ecology. Ecol. Lett. 7, 509–520. https://doi.org/10.1111/j.1461-0248.2004.00603.x

Emery, S.B., Franks, J.R., 2012. The potential for collaborative agri-environment schemes in England: Can a well-designed collaborative approach address farmers' concerns with current schemes? J. Rural Stud. 28, 218–231. https://doi.org/10.1016/j.jrurstud.2012.02.004

Ernesto Méndez, V., Bacon, C.M., Cohen, R., 2013. Agroecology as a transdisciplinary, participatory, and action-oriented approach. Agroecol. Sustain. Food Syst. https://doi.org/10.1080/10440046.2012.736926

Esperschütz, J., Gattinger, A., Mäder, P., Schloter, M., Fließbach, A., Fliessbach, A., 2007. Response of soil microbial biomass and community structures to conventional and

organic farming systems under identical crop rotations. FEMS Microbiol. Ecol. 61, 26–37. https://doi.org/10.1111/j.1574-6941.2007.00318.x

European Commission, 2020. Farm to Fork Strategy.

European Environment Agency, 2019. The European environment - state and outlook 2020: knowledge for transition to a sustainable Europe, European Environment. https://doi.org/10.2800/96749

European Parliament, 2009. Directive 2009/128/EC of the European Parliament and the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides. October 309, 71–86. https://doi.org/10.3000/17252555.L_2009.309

European Parliament, 2007. Council Directive 834/2007 on organic production and labelling of organic products. Off. J. Eur. Communities L 189 2007, 1–23.

European Union, 2020. Moving towards a more healthy and sustainable EU food system, a corner stone of the European Green Deal.

European Union, 2009. (EC) No 1107/2009. Off. J. Eur. Union 309, 1–50.

European Union, 1997. (COM)97 2000 Agenda 2000 For a stronger and wider union.

Eyre, M.D., Critchley, C.N.R., Leifert, C., Wilcockson, S.J., 2011. Crop sequence, crop protection and fertility management effects on weed cover in an organic/conventional farm management trial. Eur. J. Agron. 34, 153–162. https://doi.org/10.1016/j.eja.2011.01.001

Eyre, M.D., Labanowska-Bury, D., White, R., Leifert, C., 2010. Relationships between beneficial invertebrates, field margin vegetation, and thrip damage in organic leek fields in eastern England. Org. Agric. 1, 45–54. https://doi.org/10.1007/s13165-010-0004-x

Eyre, M D, Leifert, C., 2011. Crop and field boundary influences on the activity of a wide range of beneficial invertebrate groups on a split conventional/organic farm in northern England. Bull. Entomol. Res. 101, 135–44. https://doi.org/10.1017/S0007485310000398

Eyre, M.D., Luff, M.L., Atlihan, R., Leifert, C., 2012. Ground beetle species (Carabidae, Coleoptera) activity and richness in relation to crop type, fertility management and crop protection in a farm management comparison trial. Ann. Appl. Biol. 161, 169–179. https://doi.org/10.1111/j.1744-7348.2012.00562.x

Eyre, M.D., Luff, M.L., Leifert, C., 2013. Crop, field boundary, productivity and disturbance influences on ground beetles (Coleoptera, Carabidae) in the agroecosystem. Agric. Ecosyst. Environ. 165, 60–67. https://doi.org/10.1016/j.agee.2012.12.009

Eyre, M.D., Volakakis, N., Shotton, P.N., Leifert, C., 2007. The Effects of Crop Type and Production Systems on the Activity of Beneficial Invertebrates, in: 3rd QLIF Congress. pp. 0–4.

Eyre, M.D., Sanderson, R.A.A., Shotton, P.N.N., Leifert, C., 2009. Investigating the effects of crop type, fertility management and crop protection on the activity of beneficial invertebrates in an extensive farm management comparison trial. Ann. Appl. Biol. 155, 267–276. https://doi.org/10.1111/j.1744-7348.2009.00337.x

Fang, H., Yu, Y., Chu, X., Wang, X., Yang, X., Yu, J., 2009. Degradation of chlorpyrifos in laboratory soil and its impact on soil microbial functional diversity. J. Environ. Sci. 21, 380–386. https://doi.org/10.1016/S1001-0742(08)62280-9

FAO, 2016. Guiding the Transition To Sustainable Food and Agricultural Systems the 10 Elements of Agroecology.

FAO, 2015a. Intergovernmental Technical Panel on Soils. Status of the World's Soil Resources.

FAO, 2015b. Revised World Soil Charter 10.

FAO, 2015c. Healthy soils are the basis for healthy food production. Fao 4.

FAO, 2008. The case for improving soil health. Food Agric. Organ. United Nations - An Int. Tech. Work. Invest. Sustain. Crop intenstification 6.

FAO and WHO, 2009. How to feed the World in 2050.

Felkl, G., Jensen, E.B., Kristiansen, K., Andersen, S.B., 2005. Tolerance and antibiosis resistance to cabbage root fly in vegetable Brassica species. Entomol. Exp. Appl. 116, 65–71. https://doi.org/10.1111/j.1570-7458.2005.00312.x

Ferry, A., Dugravot, S., Delattre, T., Christides, J.-P., Auger, J., Bagnères, A.-G., Poinsot, D., Cortesero, A.-M., 2007. Identification of a widespread monomolecular odor differentially attractive to several Delia radicum ground-dwelling predators in the field. J. Chem. Ecol. 33, 2064–77. https://doi.org/10.1007/s10886-007-9373-3

Ferry, A., Le Tron, S., Dugravot, S., Cortesero, a. M.M., 2009. Field evaluation of the combined deterrent and attractive effects of dimethyl disulfide on Delia radicum and its natural enemies. Biol. Control 49, 219–226. https://doi.org/10.1016/j.biocontrol.2009.01.013

Finch, S., 1996. Effect of beetle size on predation of cabbage root fly eggs by ground beetles. Entomol. Exp. Appl. 81, 199–206. https://doi.org/10.1111/j.1570-7458.1996.tb02032.x

Finch, S., 1993. Integrated pest management of the cabbage root fly and the carrot fly. Crop Prot. 12, 423–430. https://doi.org/10.1016/0261-2194(93)90003-2

Finch, S., 1990. The effectiveness of traps used currently for monitoring populations of the cabbage root fly (Delia radicum). Ann. Appl. Biol. 116, 447–454. https://doi.org/10.1111/j.1744-7348.1990.tb06627.x

Finch, S., 1989. Ecological Considerations in the Management of Delia Pest Species in Vegetable Crops. Annu. Rev. Entomol. 34, 117–137.

https://doi.org/10.1146/annurev.en.34.010189.001001

Finch, S., Ackley, C.M., 1977. Cultivated and wild host plants supporting populations of the cabbage root fly I4. Ann. Appl. Biol. 85, 13–22.

Finch, S., Billiald, H., Collier, R.H., 2003. Companion planting - do aromatic plants disrupt host-plant finding by the cabbage root fly and the onion fly more effectively than non-aromatic plants? Entomol. Exp. Appl. 109, 183–195. https://doi.org/10.1046/j.0013-8703.2003.00102.x

Finch, S., Coaker, T.H., 1968. A method for the continuous rearing of the cabbage root fly Erioischia brassicae (Bch) and some observations on its biology 619–627.

Finch, S., Collier, R.., Skinner, G., 1986. Local population differences in emergence of cabbage root flies from south-west Lancashire: implications for pest forecasting and population divergence. Ecol. Entomol. 11, 139–145. https://doi.org/10.1111/j.1365-2311.1986.tb00288.x

Finch, S, Collier, R.H., 2000. Integrated pest management in " eld vegetable crops in northern Europe * with focus on two key pests 19, 817–824.

Finch, S., Collier, R.H., 2000. Host-plant selection by insects - a theory based on “appropriate/inappropriate landings” by pest insects of cruciferous plants. Entomol. Exp. Appl. 96, 91–102. https://doi.org/10.1046/j.1570-7458.2000.00684.x

Finch, S., Collier, R.H., 1985. Laboratory studies on aestivation in the cabbage root fly (Delia radicum). Entomol. Exp. Appl. 38, 137–143. https://doi.org/10.1111/j.1570-7458.1985.tb03510.x

Finch, S., Collier, R.H., 1984. Parasitism of overwintering pupae of cabbage root fly, Delia radicum (L.) (Diptera: Anthomyiidae), in England and Wales. Bull. Entomol. Res. 74, 79–86. https://doi.org/10.1017/S0007485300009949

Finch, S., Elliott, M.S., 1992. Carabidae as potential biological agents for controlling infestations of the cabbage root fly. Phytoparasitica 20, S67–S70. https://doi.org/10.1007/BF02980411

Finch, S., Kienegger, M., 1997. A behavioural study to help clarify how undersowing with clover affects host-plant selection by pest insects of brassica crops. Entomol. Exp. Appl. 84, 165–172. https://doi.org/10.1046/j.1570-7458.1997.00211.x

Finch, S., Skinner, G., 2009. Trapping female cabbage root flies (Delia radicum (L.)) (Diptera: Anthomyiidae) with allylisothiocyanate-Baited traps. Bull. Entomol. Res. 72, 165. https://doi.org/10.1017/S0007485300050392

Finch, S., Skinner, G., 1982. Upwind flight by the cabbage root fly, Delia radicum. Physiol. Entomol. 7, 387–399. https://doi.org/10.1111/j.1365-3032.1982.tb00314.x

Finch, S., Skinner, G., 1975. Dispersal of the cabbage root fly. Ann. Appl. Biol. 1–19.

Firbank, L., Bradbury, R.B., McCracken, D.I., Stoate, C., 2013. Delivering multiple ecosystem

services from Enclosed Farmland in the UK. Agric. Ecosyst. Environ. 166, 65–75. https://doi.org/10.1016/j.agee.2011.11.014

Firbank, L.G., Petit, S., Smart, S., Blain, A., Fuller, R.J., 2008. Assessing the impacts of agricultural intensification on biodiversity: A British perspective. Philos. Trans. R. Soc. B Biol. Sci. https://doi.org/10.1098/rstb.2007.2183

Fliessbach, A., Mader, P., 2000. Microbial biomass and size-density fractions differ between soils of organic and conventional agricultural systems. Soil Biol. Biochem. 32, 757–768.

Fließbach, A., Oberholzer, H.-R., Gunst, L., Mäder, P., 2007. Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agric. Ecosyst. Environ. 118, 273–284. https://doi.org/10.1016/j.agee.2006.05.022

Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Mueller, N.D., O'Connell, C., Ray, D.K., West, P.C., Balzer, C., Bennett, E.M., Carpenter, S.R., Hill, J., Monfreda, C., Polasky, S., Rockström, J., Sheehan, J., Siebert, S., Tilman, D., Zaks, D.P.M., 2011. Solutions for a cultivated planet. Nature 478, 337–342. https://doi.org/10.1038/nature10452

Fountain, M.T., Brown, V.K., Gange, A.C., Symondson, W.O.C., Murray, P.J., 2007. The effects of the insecticide chlorpyrifos on spider and Collembola communities ARTICLE IN PRESS. Pedobiologia (Jena). 51, 147–158. https://doi.org/10.1016/j.pedobi.2007.03.001

Fournet, S., Poinsot, D., Brunel, E., Nénon, J.P., Cortesero, A.M., 2001. Do female coleopteran parasitoids enhance their reproductive success by selecting high-quality oviposition sites? J. Anim. Ecol. 70, 1046–1052. https://doi.org/10.1046/j.0021-8790.2001.00557.x

Fournet, S., Stapel, J.O.O., Kacem, N., Nenon, J.P.P., Brunel, E., 2000. Life history comparison between two competitive Aleochara species in the cabbage root fly, Delia radicum: Implications for their use in biological control. Entomol. Exp. Appl. 96, 205–211. https://doi.org/10.1046/j.1570-7458.2000.00698.x

Francis, C., Lieblein, C., Gliessman, S., Breland, T.., Creamer, N., Harwood, R., Salomonsoon, L., Helenius, J., Rickerl, D., Salvador, R., Wiedenhoeft, M., Simmons, S., Allen, P., Altieri, M., Flora, C., Poincelot, R., 2003. Agroecology: the ecology of food systems. J. Sustain. Agric. 22, 99–118. https://doi.org/10.1300/J064v22n03

Fuller, R.J., Norton, L.R., Feber, R.E., Johnson, P.J., Chamberlain, D.E., Joys, A.C., Mathews, F., Stuart, R.C., Townsend, M.C., Manley, W.J., Wolfe, M.S., Macdonald, D.W., Firbank, L.G., 2005. Benefits of organic farming to biodiversity vary among taxa. Biol. Lett. 1, 431–434. https://doi.org/10.1098/rsbl.2005.0357

Funderburk, J.., Braxton, L.., Lynch, R.., 1990. Nontarget Effects of Soil-applied Chlorpyrifos on Defoliating Pests and Arthropod Predators in Peanut. Peanut Sci. 17, 113–117.

Furlong, M.J., 2014. Knowing your enemies: Integrating molecular and ecological methods to assess the impact of arthropod predators on crop pests. Insect Sci. https://doi.org/10.1111/1744-7917.12157

Furlong, M.J., Zalucki, M.P., 2010. Exploiting predators for pest management: The need for sound ecological assessment. Entomol. Exp. Appl. 135, 225–236. https://doi.org/10.1111/j.1570-7458.2010.00988.x

Gabriel, D., Sait, S.M., Hodgson, J.A., Schmutz, U., Kunin, W.E., Benton, T.G., 2010. Scale matters: The impact of organic farming on biodiversity at different spatial scales. Ecol. Lett. 13, 858–869. https://doi.org/10.1111/j.1461-0248.2010.01481.x

Gagic, V., Bartomeus, I., Jonsson, T., Taylor, A., Winqvist, C., Fischer, C., Slade, E.M., Steffan-Dewenter, I., Emmerson, M., Potts, S.G., Tscharntke, T., Weisser, W., Bommarco, R., 2015. Functional identity and diversity of animals predict ecosystem functioning better than species-based indices. Proc. R. Soc. B Biol. Sci. 282. https://doi.org/10.1098/rspb.2014.2620

Gardarin, A., Plantegenest, M., Bischoff, A., Valantin-Morison, M., 2018. Understanding plant–arthropod interactions in multitrophic communities to improve conservation biological control: useful traits and metrics. J. Pest Sci. (2004). 91, 943–955. https://doi.org/10.1007/s10340-018-0958-0

Gardi, C., Montanarella, L., Arrouays, D., Bispo, a., Lemanceau, P., Jolivet, C., Mulder, C., Ranjard, L., Römbke, J., Rutgers, M., Menta, C., 2009. Soil biodiversity monitoring in Europe: ongoing activities and challenges. Eur. J. Soil Sci. 60, 807–819. https://doi.org/10.1111/j.1365-2389.2009.01177.x

Garibaldi, L.A., Gemmill-Herren, B., D'Annolfo, R., Graeub, B.E., Cunningham, S.A., Breeze, T.D., 2017. Farming Approaches for Greater Biodiversity, Livelihoods, and Food Security. Trends Ecol. Evol. 32, 68–80. https://doi.org/10.1016/j.tree.2016.10.001

Garibaldi, L.A., Pérez-Méndez, N., Garratt, M.P.D., Gemmill-Herren, B., Miguez, F.E., Dicks, L. V., 2019. Policies for Ecological Intensification of Crop Production. Trends Ecol. Evol. 34, 282–286. https://doi.org/10.1016/j.tree.2019.01.003

Garnett, P., Doherty, B., Heron, T., 2020. Vulnerability of the United Kingdom's food supply chains exposed by COVID-19. Nat. Food 1, 315–318. https://doi.org/10.1038/s43016-020-0097-7

Garratt, M.P.D., Wright, D.J., Leather, S.R., 2011. The effects of farming system and fertilisers on pests and natural enemies: A synthesis of current research. Agric. Ecosyst. Environ. 141, 261–270. https://doi.org/10.1016/j.agee.2011.03.014

Geiger, F., Bengtsson, J., Berendse, F., Weisser, W.W., Emmerson, M., Morales, M.B., Ceryngier, P., Liira, J., Tscharntke, T., Winqvist, C., Eggers, S., Bommarco, R., Pärt, T., Bretagnolle, V., Plantegenest, M., Clement, L.W., Dennis, C., Palmer, C., Oñate, J.J., Guerrero, I., Hawro, V., Aavik, T., Thies, C., Flohre, A., Hänke, S., Fischer, C., Goedhart, P.W., Inchausti, P., 2010. Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. Basic Appl. Ecol. 11, 97–105. https://doi.org/10.1016/j.baae.2009.12.001

Gemmill-Herren, B., 2020. Closing the circle: an agroecological response to covid-19. Agric. Human Values. https://doi.org/10.1007/s10460-020-10097-7

Ghaley, B.B., Rusu, T., Sandén, T., Spiegel, H., Menta, C., Visioli, G., O'Sullivan, L., Gattin, I.T., Delgado, A., Liebig, M.A., Vrebos, D., Szegi, T., Michéli, E., Cacovean, H., Henriksen, C.B., 2018. Assessment of benefits of conservation agriculture on soil functions in arable production systems in Europe. Sustain. 10. https://doi.org/10.3390/su10030794

Gliessman, S.R., 2015. Agroecology for Food Security and Nutrition: Proceedings of the FAO International Symposium Rome 2015, Agroecology for food security and nutrition: Proceedings of the FAO international symposium.

Gliessman, S.R., 2016. Agroecology and Agroecosystems 19–29. https://doi.org/10.2134/agronmonogr43.c2

Gliessman, S.R., 1990. Agroecology: Researching the Ecological Basis for Sustainable Agriculture. Springer, New York, NY, pp. 3–10. https://doi.org/10.1007/978-1-4612-3252-0_1

Gliessman, S.R., Engles, E., Krieger, R., 1998. Agroecology: Ecological Processes in Sustainable Agriculture. CRC Press.

Goble, T.A., Dames, J.F., Hill, M.P., Moore, S.D., 2010. The effects of farming system, habitat type and bait type on the isolation of entomopathogenic fungi from citrus soils in the Eastern Cape Province, South Africa. BioControl 55, 399–412. https://doi.org/10.1007/s10526-009-9259-0

Gontijo, L.M., 2018. Engineering natural enemy shelters to enhance conservation biological control in field crops. Biol. Control 130, 155–163. https://doi.org/10.1016/J.BIOCONTROL.2018.10.014

Gouinguené, S., Poiger, T., Städler, E., 2006. Eggs of cabbage root fly stimulate conspecific oviposition: Evaluation of the activity and determination of an egg-associated compound. Chemoecology 16, 107–113. https://doi.org/10.1007/s00049-006-0335-y

Gouinguené, S., Städler, E., 2006. Comparison of the egg-laying behaviour and electrophysiological responses of Delia radicum and Delia floralis to cabbage leaf compounds. Physiol. Entomol. 31, 382–389. https://doi.org/10.1111/j.1365-3032.2006.00532.x

Grewal, P.S., Lewis, E.E., Gaugler, R., Campbell, J.F., 2009. Host finding behaviour as a predictor of foraging strategy in entomopathogenic nematodes. Parasitology 108, 207. https://doi.org/10.1017/S003118200006830X

Gunton, R.M., Firbank, L.G., Inman, A., Winter, D.M., 2016. How scalable is sustainable intensification? Nat. Plants 2, 1–4. https://doi.org/10.1038/NPLANTS.2016.65

Gurr, G.M., Wratten, S.., Snyder, W.. (Eds.), 2012. Biodiversity and insect pests: Key issues for sustainable management, 2012th ed. Wiley Blackwell.

Gurr, G.M., Lu, Z., Zheng, X., Xu, H., Zhu, P., Chen, G., Yao, X., Cheng, J., Zhu, Z., Catindig, J.L., Villareal, S., Van Chien, H., Cuong, L.Q., Channoo, C., Chengwattana, N., Lan, L.P., Hai, L.H., Chaiwong, J., Nicol, H.I., Perovic, D.J., Wratten, S.D., Heong, K.L., 2016. Multi-

country evidence that crop diversification promotes ecological intensification of agriculture. Nat. Plants 2, 22–25. https://doi.org/10.1038/NPLANTS.2016.14

Gurr, G.M., Scarratt, S.L., Wratten, S.D., Berndt, L., Irvin, N., 2004. Ecological Engineering for Pest Management—Advances in Habitat Manipulation for Arthropods. CSIRO publishing. https://doi.org/10.1663/0013-0001(2005)059[0299:dfabre]2.0.co;2

Gurr, G.M., van Emden, H.F., Wratten, S., 1998. 09- Habitat manipulation and natural enemy efficiency: implications for the control of pests. Conserv. Biol. Control 155–183. https://doi.org/10.1016/B978-012078147-8/50055-4

Gurr, G.M., Wratten, S.D. (Eds.), 2000a. Biological Control : Measures of Success. Springer.

Gurr, G.M., Wratten, S.D., Altieri, M.A., 2004. Ecological engineering : a new direction for agricultural pest management 1, 28–35.

Gurr, G.M., Wratten, S.D., Luna, J.M., 2003. Multi-function agricultural biodiversity: pest management and other benefits. Basic Appl. Ecol. 4, 107–116. https://doi.org/10.1078/1439-1791-00122

Gurr, G.M., Wratten, S.D., Landis, D.A., You, M., Wratten, S.D., Gurr, G.M., 2000b. Habitat Management to Suppress Pest Populations: Progress and Prospects. Annu. Rev. Entomol. 62, 175–201. https://doi.org/10.1146/annurev-ento-031616-035050

Gurr, G.M., You, M., 2015. Conservation Biological Control of Pests in the Molecular Era: New Opportunities to Address Old Constraints. Front. Plant Sci. 6, 1255. https://doi.org/10.3389/fpls.2015.01255

Häffner, E., Diederichsen, E., 2016. Belowground Defence Strategies Against Verticillium Pathogens. https://doi.org/10.1007/978-3-319-42319-7_6

Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D., de Kroon, H., 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS One 12, e0185809. https://doi.org/10.1371/journal.pone.0185809

Halsal, N.B., Wratten, S.D., 1988. The efficiency of pitfall trapping for polyphagous predatory Carabidae. Ecol. Entomol. 13, 293–299. https://doi.org/10.1111/j.1365-2311.1988.tb00359.x

Hancock, M.H., Legg, C.J., 2012. Pitfall trapping bias and arthropod body mass. Insect Conserv. Divers. 5, 312–318. https://doi.org/10.1111/j.1752-4598.2011.00162.x

Hartmann, M., Fliessbach, A., Oberholzer, H.-R.R., Widmer, F., 2006. Ranking the magnitude of crop and farming system effects on soil microbial biomass and genetic structure of bacterial communities. FEMS Microbiol. Ecol. 57, 378–388. https://doi.org/10.1111/j.1574-6941.2006.00132.x

Hartmann, M., Frey, B., Mayer, J., Mäder, P., Widmer, F., 2015. Distinct soil microbial diversity under long-term organic and conventional farming. ISME J. 9, 1177–1194. https://doi.org/10.1038/ismej.2014.210

Harwood, J.D., Obrycki, J.J., 2005. Quantifying aphid predation rates of generalist predators in the field. Eur. J. Entomol. 102, 335–350. https://doi.org/10.14411/eje.2005.051

Harwood, J.D., Phillips, S.W., Lello, J., Sunderland, K.D., Glen, D.M., Bruford, M.W., Harper, G.L., Symondson, W.O.C.C., 2009. Invertebrate biodiversity affects predator fitness and hence potential to control pests in crops. Biol. Control 51, 499–506. https://doi.org/10.1016/j.biocontrol.2009.09.007

Hathaway-Jenkins, L.J., Sakrabani, R., Pearce, B., Whitmore, A.P., Godwin, R.J., 2011. A comparison of soil and water properties in organic and conventional farming systems in England. Soil Use Manag. 27, 133–142. https://doi.org/10.1111/j.1475-2743.2011.00335.x

Havukkala, I., 1988. Non-chemical control methods against cabbage root flies Delia radicum and Delia floralis (Anthomyiidae). Ann. Agric. Fenn. 27, 271–1988.

Hawes, C., Haughton, A.J., Bohan, D.A., Squire, G.R., 2009. Functional approaches for assessing plant and invertebrate abundance patterns in arable systems. Basic Appl. Ecol. 10, 34–42. https://doi.org/10.1016/j.baae.2007.11.007

Haygarth, P.M., Ritz, K., 2009. The future of soils and land use in the UK: Soil systems for the provision of land-based ecosystem services. Land use policy 26, S187–S197. https://doi.org/10.1016/j.landusepol.2009.09.016

Hazell, P., Wood, S., 2008. Drivers of change in global agriculture. Philos. Trans. R. Soc. B Biol. Sci. 363, 495–515. https://doi.org/10.1098/rstb.2007.2166

Heimoana, V., Pilkington, L.J., Raman, A., Mitchell, A., Nicol, H.I., Johnson, A.C., Gurr, G.M., 2017. Integrating spatially explicit molecular and ecological methods to explore the significance of non-crop vegetation to predators of brassica pests. Agric. Ecosyst. Environ. 239, 12–19. https://doi.org/10.1016/j.agee.2017.01.008

Hemachandra, K.S.S., Holliday, N.J.J., Mason, P.G.G., Soroka, J.J.J., Kuhlmann, U., 2007. Comparative assessment of the parasitoid community of Delia radicum in the Canadian prairies and Europe: A search for classical biological control agents. Biol. Control 43, 85–94. https://doi.org/10.1016/j.biocontrol.2007.07.005

Henneron, L., Bernard, L., Hedde, M., Pelosi, C., Villenave, C., Chenu, C., Bertrand, M., Girardin, C., Blanchart, E., 2014. Fourteen years of evidence for positive effects of conservation agriculture and organic farming on soil life. Agron. Sustain. Dev. 35, 169–181. https://doi.org/10.1007/s13593-014-0215-8

Herbst, M., Razinger, J., Ugrinović, K., Škof, M., Schroers, H.-J.J., Hommes, M., Poehling, H.-M.M., 2017. Evaluation of low risk methods for managing Delia radicum, cabbage root fly, in broccoli production. Crop Prot. 96, 273–280. https://doi.org/10.1016/j.cropro.2017.02.023

Hernández-Hierro, J.M., Valverde, J., Villacreces, S., Reilly, K., Gaffney, M., González-Miret, M.L., Heredia, F.J., Downey, G., 2012. Feasibility study on the use of visible-near-infrared spectroscopy for the screening of individual and total glucosinolate contents in broccoli. J. Agric. Food Chem. 60, 7352–7358. https://doi.org/10.1021/jf3018113

Hillocks, R.J., 2012. Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. Crop Prot. 31, 85–93. https://doi.org/10.1016/j.cropro.2011.08.008

Hobbs, J.E., 2020. Food supply chains during the COVID-19 pandemic. Can. J. Agric. Econ. 1–6. https://doi.org/10.1111/cjag.12237

Hobbs, P.J., Webb, J., Mottram, T.T., Grant, B., Misselbrook, T.M., 2004. Emissions of volatile organic compounds originating from UK livestock agriculture. J. Sci. Food Agric. 84, 1414–1420. https://doi.org/10.1002/jsfa.1810

Hofsvang, T., 1991. The influence of intercropping and weeds on the oviposition of the brassica root flies (Delia radicum and D. floralis). Nor. J. Agric. Sci. 5, 349–356.

Hole, D.G., Perkins, A.J., Wilson, J.D., Alexander, I.H., Grice, P.V., Evans, a. D., 2005. Does organic farming benefit biodiversity? Biol. Conserv. 122, 113–130. https://doi.org/10.1016/j.biocon.2004.07.018

Holland, J.M., 2012. Promoting agri-environment schemes for conservation biocontrol. IOBC (International Organ. Biol. Integr. Control. West Palearct. Reg. Sect. Bull. 75. Landsc, 99–103.

Holland, J.M., Bianchi, F.J., Entling, M.H., Moonen, A.-C.C., Smith, B.M., Jeanneret, P., 2016. Structure, function and management of semi-natural habitats for conservation biological control: a review of European studies. Pest Manag. Sci. 72, 1638–1651. https://doi.org/10.1002/ps.4318

Holland, J.M., Douma, J.C., Crowley, L., James, L., Kor, L., Stevenson, D.R.W., Smith, B.M., 2017. Semi-natural habitats support biological control, pollination and soil conservation in Europe. A review. Agron. Sustain. Dev. 37. https://doi.org/10.1007/s13593-017-0434-x

Holland, J.M., Jeanneret, P., Moonen, A., Werf, W. Van Der, Rossing, W.A.H., Antichi, D., Entling, M.H., Gi, B., Helsen, H., Szalai, M., Rega, C., Gibert, C., Veromann, E., 2020. Approaches to Identify the Value of Seminatural Habitats for Conservation Biological Control. Insects 11, 1–11.

Holland, J.M., Oakley, J., 2007. Importance of arthropod pests and their natural enemies in relation to recent farming practice changes in the UK.

Holland, J.M., Perry, J.N., Winder, L., Faculty, U.K.S., Abbot, N., 1999. The within-field spatial and temporal distribution of arthropods in winter wheat 499–513.

Holt-Giménez, E., Altieri, M.A., 2013. Agroecology, food sovereignty, and the new green revolution. Agroecol. Sustain. Food Syst. 37, 90–102. https://doi.org/10.1080/10440046.2012.716388

Hominick, W.M., Briscoe, B.R., 1990. Occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in British soils. Parasitology 100, 295–302. https://doi.org/10.1017/S0031182000061308

Hooks, C.R.R., Johnson, M.W., 2003. Impact of agricultural diversification on the insect community of cruciferous crops. Crop Prot. 22, 223–238.

Hooper, D.U., Adair, E.C., Cardinale, B.J., Byrnes, J.E.K., Hungate, B.A., Matulich, K.L., Gonzalez, A., Duffy, J.E., Gamfeldt, L., Connor, M.I., 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature 486, 105–108. https://doi.org/10.1038/nature11118

Hopkins, R.J., Griffiths, D.W., Birch, A.N.E., McKinlay, R.G., 1998. Influence of Increasing Herbivore Pressure on Modification of Glucosinolate Content of Swedes (Brassica napus spp. rapifera). J. Chem. Ecol. 24, 2003–2019. https://doi.org/10.1023/A:1020729524818

Hopkins, R.J., van Dam, N.M., van Loon, J.J. a, 2009. Role of glucosinolates in insect-plant relationships and multitrophic interactions. Annu. Rev. Entomol. 54, 57–83. https://doi.org/10.1146/annurev.ento.54.110807.090623

Hoy, C.W., Grewal, P.S., Lawrence, J.L., Jagdale, G., Acosta, N., 2008. Canonical correspondence analysis demonstrates unique soil conditions for entomopathogenic nematode species compared with other free-living nematode species. Biol. Control 46, 371–379. https://doi.org/10.1016/j.biocontrol.2008.06.001

Hughes, R.D., Salter, D.D., 1959. Natural Mortality of Erioischia brassicae (Bouche) (Diptera, Anthomyiidae) During the Immature Stages of the First Generation. J. Anim. Ecol. 28, 231. https://doi.org/10.2307/2080

Huguenin, M.T., Leggett, C.G., Paterson, R.W., 2006. Economic valuation of soil fauna. Eur. J. Soil Biol. 42, S16–S22. https://doi.org/10.1016/j.ejsobi.2006.10.003

Hummel, J.D., Dosdall, L.M., Clayton, G.W., Harker, K.N., O'Donovan, J.T., O'Donovan, J.T., 2010. Responses of the parasitoids of Delia radicum (Diptera: Anthomyiidae) to the vegetational diversity of intercrops. Biol. Control 55, 151–158. https://doi.org/10.1016/j.biocontrol.2010.08.004

Hummel, R.L., Walgenbach, J.F., Barbercheck, M.E., Kennedy, G.G., Hoyt, G.D., Arellano, C., 2002. Effects of Production Practices on Soil-Borne Entomopathogens in Western North Carolina Vegetable Systems. Environ. Entomol. 31, 84–91. https://doi.org/10.1603/0046-225x-31.1.84

Hummel, R.L, Walgenbach, J.F., Hoyt, G.D., Kennedy, G.G., 2002a. Effects of vegetable production system on epigeal arthropod populations 93, 177–188.

Hummel, R.L, Walgenbach, J.F., Hoyt, G.D., Kennedy, G.G., 2002b. Effects of production system on vegetable arthropods and their natural enemies 93, 165–176.

Hunter, M.C., Smith, R.G., Schipanski, M.E., Atwood, L.W., Mortensen, D.A., 2017. Agriculture in 2050: Recalibrating targets for sustainable intensification. Bioscience 67, 386–391. https://doi.org/10.1093/biosci/bix010

Hurlbert, S., 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54, 187–211.

IAASTD, 2009. Agriculture at a crossroads. Synthesis Report., International Assessment of Agricultural Knowledge, Science, and Technology for Development.

IFOAM, 2014. Organic agriculture principles.

IPES-Food, 2015. IPES-Food : 10 Principles to guide the transition to Sustainable Food Systems. Int. Panel Expert. Sustain. Food Syst.

IRAG, 2019. Insecticide resistance status in UK oilseed rape crops 1–4.

Jabbour, R., Barbercheck, M.E., 2009. Soil management effects on entomopathogenic fungi during the transition to organic agriculture in a feed grain rotation. Biol. Control 51, 435–443. https://doi.org/10.1016/j.biocontrol.2009.08.004

Jacobsen, S.K., Moraes, G.J., Sørensen, H., Sigsgaard, L., 2019. Organic cropping practice decreases pest abundance and positively influences predator-prey interactions. Agric. Ecosyst. Environ. 272, 1–9. https://doi.org/10.1016/J.AGEE.2018.11.004

Jaffuel, G., Mäder, P., Blanco-Perez, R., Chiriboga, X., Fliessbach, A., Turlings, T.C.J., Campos-Herrera, R., 2016. Prevalence and activity of entomopathogenic nematodes and their antagonists in soils that are subject to different agricultural practices. Agric. Ecosyst. Environ. 230, 329–340. https://doi.org/10.1016/j.agee.2016.06.009

Jeanneret, P., Begg, G., Gosme, M., Alomar, O., Reubens, B., Baudry, J., Guerin, O., Flamm, C., Wäckers, F., 2016. Landscape Features to Improve Pest Control in Agriculture. Solutions 7, 48–57.

Jensen, E.B., Felkl, G., Kristiansen, K., Andersen, S.B., 2002. Resistance to the cabbage root fly, Delia radicum, within Brassica fruticulosa. Euphytica 124, 379–386. https://doi.org/10.1023/A:1015755306547

Jonsson, M., Wratten, S.D., Landis, D.A., Gurr, G.M., 2008. Recent advances in conservation biological control of arthropods by arthropods. Biol. Control 45, 172–175. https://doi.org/10.1016/j.biocontrol.2008.01.006

Josso, C., Le Ralec, A., Raymond, L., Saulais, J., Baudry, J., Poinsot, D., Cortesero, A.M., 2013. Effects of field and landscape variables on crop colonization and biological control of the cabbage root fly Delia radicum. Landsc. Ecol. 28, 1697–1715. https://doi.org/10.1007/s10980-013-9928-3

Jowett, K., Milne, A.E., Metcalfe, H., Hassall, K.L., Potts, S.G., Senapathi, D., Storkey, J., 2019. Species matter when considering landscape effects on carabid distributions. Agric. Ecosyst. Environ. 285, 106631. https://doi.org/10.1016/J.AGEE.2019.106631

Jyoti, J.L., Shelton, a M., Earle, E.D., 2001. Identifying sources and mechanisms of resistance in crucifers for control of cabbage maggot (Diptera: Anthomyiidae). J. Econ. Entomol. 94, 942–9.

Kabouw, P., Kos, M., Kleine, S., Vockenhuber, E. a. A., van Loon, J.J. a. J.A., van der Putten, W.H.H., Van Dam, N.M.M., Biere, a., 2011. Effects of soil organisms on aboveground multitrophic interactions are consistent between plant genotypes mediating the

interaction. Entomol. Exp. Appl. 139, 197–206. https://doi.org/10.1111/j.1570-7458.2011.01123.x

Kada, H.O., Ba, H.O., 2008. Comparison of nematode community similarities assessed by polymerase chain reaction-denaturing gradient gel electrophoresis (DGGE) and by morphological identification 10, 689–700.

Karp, D.S., Chaplin-Kramer, R., Meehan, T.D., Martin, E.A., DeClerck, F., Grab, H., Gratton, C., Hunt, L., Larsen, A.E., Martínez-Salinas, A., O'Rourke, M.E., Rusch, A., Poveda, K., Jonsson, M., Rosenheim, J.A., Schellhorn, N.A., Tscharntke, T., Wratten, S.D., Zhang, W., Iverson, A.L., Adler, L.S., Albrecht, M., Alignier, A., Angelella, G.M., Anjum, M.Z., Avelino, J., Batáry, P., Baveco, J.M., Bianchi, F.J.J.A.J.A., Birkhofer, K., Bohnenblust, E.W., Bommarco, R., Brewer, M.J., Caballero-López, B., Carrière, Y., Carvalheiro, L.G., Cayuela, L., Centrella, M., Ćetković, A., Henri, D.C., Chabert, A., Costamagna, A.C., De la Mora, A., de Kraker, J., Desneux, N., Diehl, E., Diekötter, T., Dormann, C.F., Eckberg, J.O., Entling, M.H., Fiedler, D., Franck, P., van Veen, F.J.F., Frank, T., Gagic, V., Garratt, M.P.D.D., Getachew, A., Gonthier, D.J., Goodell, P.B., Graziosi, I., Groves, R.L., Gurr, G.M., Hajian-Forooshani, Z., Heimpel, G.E., Herrmann, J.D., Huseth, A.S., Inclán, D.J., Ingrao, A.J., Iv, P., Jacot, K., Johnson, G.A., Jones, L., Kaiser, M., Kaser, J.M., Keasar, T., Kim, T.N., Kishinevsky, M., Landis, D.A., Lavandero, B., Lavigne, C., Le Ralec, A., Lemessa, D., Letourneau, D.K., Liere, H., Lu, Y., Lubin, Y., Luttermoser, T., Maas, B., Mace, K., Madeira, F., Mader, V., Cortesero, A.M., Marini, L., Martinez, E., Martinson, H.M., Menozzi, P., Mitchell, M.G.E.E., Miyashita, T., Molina, G.A.R.R., Molina-Montenegro, M.A., O'Neal, M.E., Opatovsky, I., Ortiz-Martinez, S., Nash, M., Östman, Ö., Ouin, A., Pak, D., Paredes, D., Parsa, S., Parry, H., Perez-Alvarez, R., Perović, D.J., Peterson, J.A., Petit, S., Philpott, S.M., Plantegenest, M., Plećas, M., Pluess, T., Pons, X., Potts, S.G., Pywell, R.F., Ragsdale, D.W., Rand, T.A., Raymond, L., Ricci, B., Sargent, C., Sarthou, J.-P.P., Saulais, J., Schäckermann, J., Schmidt, N.P., Schneider, G., Schüepp, C., Sivakoff, F.S., Smith, H.G., Whitney, K.S., Stutz, S., Szendrei, Z., Takada, M.B., Taki, H., Tamburini, G., Thomson, L.J., Tricault, Y., Tsafack, N., Tschumi, M., Valantin-Morison, M., Van Trinh, M., van der Werf, W., Vierling, K.T., Werling, B.P., Wickens, J.B., Wickens, V.J., Woodcock, B.A., Wyckhuys, K., Xiao, H., Yasuda, M., Yoshioka, A., Zou, Y., O'Rourke, M.E., Rusch, A., Poveda, K., Jonsson, M., Rosenheim, J.A., Schellhorn, N.A., Tscharntke, T., Wratten, S.D., Zhang, W., Iverson, A.L., Adler, L.S., Albrecht, M., Alignier, A., Angelella, G.M., Zubair Anjum, M., Avelino, J., Batáry, P., Baveco, J.M., Bianchi, F.J.J.A.J.A., Birkhofer, K., Bohnenblust, E.W., Bommarco, R., Brewer, M.J., Caballero-López, B., Carrière, Y., Carvalheiro, L.G., Cayuela, L., Centrella, M., Ćetković, A., Henri, D.C., Chabert, A., Costamagna, A.C., De la Mora, A., de Kraker, J., Desneux, N., Diehl, E., Diekötter, T., Dormann, C.F., Eckberg, J.O., Entling, M.H., Fiedler, D., Franck, P., Frank van Veen, F.J., Frank, T., Gagic, V., Garratt, M.P.D.D., Getachew, A., Gonthier, D.J., Goodell, P.B., Graziosi, I., Groves, R.L., Gurr, G.M., Hajian-Forooshani, Z., Heimpel, G.E., Herrmann, J.D., Huseth, A.S., Inclán, D.J., Ingrao, A.J., Iv, P., Jacot, K., Johnson, G.A., Jones, L., Kaiser, M., Kaser, J.M., Keasar, T., Kim, T.N., Kishinevsky, M., Landis, D.A., Lavandero, B., Lavigne, C., Le Ralec, A., Lemessa, D., Letourneau, D.K., Liere, H., Lu, Y., Lubin, Y., Luttermoser, T., Maas, B., Mace, K., Madeira, F., Mader, V., Cortesero, A.M., Marini, L., Martinez, E., Martinson, H.M., Menozzi, P., Mitchell, M.G.E.E., Miyashita, T., Molina, G.A.R.R., Molina-Montenegro, M.A., O'Neal, M.E., Opatovsky, I., Ortiz-Martinez, S., Nash, M., Östman, Ö., Ouin, A., Pak, D., Paredes, D., Parsa, S., Parry, H., Perez-Alvarez, R., Perović, D.J., Peterson, J.A., Petit, S., Philpott, S.M., Plantegenest, M., Plećaš, M., Pluess, T., Pons, X., Potts, S.G.,

Pywell, R.F., Ragsdale, D.W., Rand, T.A., Raymond, L., Ricci, B., Sargent, C., Sarthou, J.-P.P., Saulais, J., Schäckermann, J., Schmidt, N.P., Schneider, G., Schüepp, C., Sivakoff, F.S., Smith, H.G., Stack Whitney, K., Stutz, S., Szendrei, Z., Takada, M.B., Taki, H., Tamburini, G., Thomson, L.J., Tricault, Y., Tsafack, N., Tschumi, M., Valantin-Morison, M., Van Trinh, M., van der Werf, W., Vierling, K.T., Werling, B.P., Wickens, J.B., Wickens, V.J., Woodcock, B.A., Wyckhuys, K., Xiao, H., Yasuda, M., Yoshioka, A., Zou, Y., 2018. Crop pests and predators exhibit inconsistent responses to surrounding landscape composition. Proc. Natl. Acad. Sci. U. S. A. 115, E7863–E7870. https://doi.org/10.1073/pnas.1800042115

Kaya, H.K., Gaugler, R., 1993. Entomopathogenic nematodes. Annu. Rev. Entomol. 38, 181–206.

Kaya, H.K., Koppenhöfer, A.M., 1996. Effects of microbial and other antagonistic organism and competition on entomopathogenic nematodes. Biocontrol Sci. Technol. 6, 357–372. https://doi.org/10.1080/09583159631334

Kaya, H.K., Vega, F.E., 2012. Scope and basic principles of insect pathology, Second Edi. ed, Insect Pathology. Elsevier Inc. https://doi.org/10.1016/B978-0-12-384984-7.00001-4

Keating, M., 2019. The Repatriation of Competences in Agriculture after Brexit.

Kergunteuil, a., Cortesero, a. M., Chaminade, V., Dourlot, S., Paty, C., Le Ralec, a., Dugravot, S., 2014. Field and laboratory selection of brassicaceous plants that differentially affect infestation levels by Delia radicum. J. Appl. Entomol. n/a-n/a. https://doi.org/10.1111/jen.12187

Kergunteuil, A., Dugravot, S., Mortreuil, A., Le Ralec, A., Cortesero, A.M., 2012. Selecting volatiles to protect brassicaceous crops against the cabbage root fly, Delia radicum. Entomol. Exp. Appl. 144, 69–77. https://doi.org/10.1111/j.1570-7458.2012.01257.x

Kessler, P., Matzke, H., Keller, S., 2003. The effect of application time and soil factors on the occurrence of Beauveria brongniartii applied as a biological control agent in soil. J. Invertebr. Pathol. 84, 15–23. https://doi.org/10.1016/j.jip.2003.08.003

Klingen, I., Eilenberg, J., Meadow, R., 2002. Effects of farming system, field margins and bait insect on the occurrence of insect pathogenic fungi in soils. Agric. Ecosyst. Environ. 91, 191–198. https://doi.org/10.1016/S0167-8809(01)00227-4

Klingen, I., Haukeland, S., 2007. The soil as a reservoir for natural enemies of pest insects and mites with emphasis on fungi and nematodes, in: An Ecological and Societal Approach to Biological Control. Springer Netherlands, pp. 145–211. https://doi.org/10.1007/978-1-4020-4401-4_9

Klingen, I., Haukeland, S., 2006. THE SOIL AS A RESERVOIR FOR NATURAL ENEMIES OF AND NEMATODES.

Klingen, I., Meadow, R., Aandal, T., 2002. Mortality of Delia floralis, Galleria mellonella and Mamestra brassicae treated with insect pathogenic hyphomycetous fungi. J. Appl. Entomol. 126, 231–237. https://doi.org/10.1046/j.1439-0418.2002.00636.x

Klingen, I., Meadow, R., Eilenberg, J., 2000. Prevalence of fungal infections in adult Delia radicum and Delia floralis captured on the edge of a cabbage field 265–274.

Kogan, M., 1998. Integrated Pest Management: Historical Perspectives and Contemporary Developments. Annu. Rev. Entomol. 43, 243–270. https://doi.org/10.1146/annurev.ento.43.1.243

Koppenhöfer, A.M., Fuzy, E.M., 2006. Effect of soil type on infectivity and persistence of the entomopathogenic nematodes Steinernema scarabaei, Steinernema glaseri, Heterorhabditis zealandica, and Heterorhabditis bacteriophora. J. Invertebr. Pathol. 92, 11–22. https://doi.org/10.1016/j.jip.2006.02.003

Koppenhöfer, A.M., Kaya, H.K., 1997. Additive and synergistic interaction between entomopathogenic nematodes and Bacillus thuringiensis for scarab grub control. Biol. Control 8, 131–137. https://doi.org/10.1006/bcon.1996.0498

Kostal, V., Simek, P., 1995. Dynamics of Cold Hardiness , Supercooling and Cryoprotectants in Diapausing and Non-diapausing Pupae of the Cabbage Root Fly , Delia radicum L . J. Insect Physiol. 41, 627–634.

Krebs, J.R., Wilson, J.D., Bradbury, R.B., Siriwardena, G.M., 1999. The second silent spring? Nature 400, 611–612. https://doi.org/10.1038/23127

Kupferschmied, P., Maurhofer, M., Keel, C., 2013. Promise for plant pest control: root-associated pseudomonads with insecticidal activities. Front. Plant Sci. 4, 287. https://doi.org/10.3389/fpls.2013.00287

Lacey, L., Frutos, R., Kaya, H., Vail, P., 2001. Insect Pathogens as Biological Control Agents: Do They Have a Future? Biol. Control 21, 230–248. https://doi.org/10.1006/bcon.2001.0938

Lacey, L., 2012. Manual of Techniques in invertebrate pathology, 2nd ed. Academic press.

Lacey, L.A., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M., Goettel, M.S., 2015. Insect pathogens as biological control agents: Back to the future. J. Invertebr. Pathol. 132, 1–41. https://doi.org/10.1016/j.jip.2015.07.009

Lachaise, T., Ourry, M., Lebreton, L., Guillerm-Erckelboudt, A.-Y., Linglin, J., Paty, C., Chaminade, V., Marnet, N., Aubert, J., Poinsot, D., Cortesero, A.-M., Mougel, C., 2017. Can soil microbial diversity influence plant metabolites and life history traits of a rhizophagous insect? A demonstration in oilseed rape. Insect Sci. https://doi.org/10.1111/1744-7917.12478

Lamy, F., Bellec, L., Rusu-stievenard, A., Clin, P., Ricono, C., Olivier, D., Poinsot, D., Faloya, V., Daniel, L., Cortesero, A.M., 2020. Oviposition Preference of the Cabbage Root Fly towards Some Chinese Cabbage Cultivars : A Search.

Lamy, F., Dugravot, S., Cortesero, A.M., Chaminade, V., Faloya, V., Poinsot, D., 2018. One more step toward a push-pull strategy combining both a trap crop and plant volatile organic compounds against the cabbage root fly Delia radicum. Environ. Sci. Pollut. Res. 25, 29868–29879. https://doi.org/10.1007/s11356-017-9483-6

Landis, D.A., Wratten, S.D., Gurr, G.M., 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annu. Rev. Entomol. 45, 175–201. https://doi.org/10.1146/annurev.ento.45.1.175

Lang, A., 2000. The pitfalls of pitfalls : a comparison of pitfall trap catches and absolute density estimates of epigeal invertebrate predators in arable land. J. Pest Sci. (2004). 106, 99–106.

Langlet, X., Brunel, E., 1996. Preliminary study on predation by Aleochara bilineata Gyll. (Coleoptera, Staphylinidae). IOBC wrps Bull. 19, 173–178.

Lavelle, P., Decaëns, T., Aubert, M., Barot, S., Blouin, M., Bureau, F., Margerie, P., Mora, P., Rossi, J.P., 2006. Soil invertebrates and ecosystem services. Eur. J. Soil Biol. 42, S3–S15. https://doi.org/10.1016/j.ejsobi.2006.10.002

Le Campion, A., Oury, F.X., Heumez, E., Rolland, B., 2020. Conventional versus organic farming systems: dissecting comparisons to improve cereal organic breeding strategies. Org. Agric. 10, 63–74. https://doi.org/10.1007/s13165-019-00249-3

Lechenet, M., Dessaint, F., Py, G., Makowski, D., Munier-Jolain, N., 2017. Reducing pesticide use while preserving crop productivity and profitability on arable farms. Nat. Plants 3, 1–6. https://doi.org/10.1038/nplants.2017.8

Lefcheck, J.S., Byrnes, J.E.K., Isbell, F., Gamfeldt, L., Griffin, J.N., Eisenhauer, N., Hensel, M.J.S., Hector, A., Cardinale, B.J., Duffy, J.E., 2015. Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats. Nat. Commun. 6. https://doi.org/10.1038/ncomms7936

Leger, C., Riga, E., 2009. Evaluation of Marigolds and Entomopathogenic Nematodes for Control of the Cabbage Maggot Delia radicum. J. Sustain. Agric. 33, 128–141. https://doi.org/10.1080/10440040802394992

Lemanceau, P., Maron, P.A., Mazurier, S., Mougel, C., Pivato, B., Plassart, P., Ranjard, L., Revellin, C., Tardy, V., Wipf, D., 2014. Understanding and managing soil biodiversity: a major challenge in agroecology. Agron. Sustain. Dev. 35, 67–81. https://doi.org/10.1007/s13593-014-0247-0

Lescourret, F., Dutoit, T., Rey, F., Côte, F., Hamelin, M., Lichtfouse, E., 2015. Agroecological engineering. Agron. Sustain. Dev. https://doi.org/10.1007/s13593-015-0335-9

Letourneau, D.K., Bothwell, S.G., 2008. Comparison of organic and conventional farms: Challenging ecologists to make biodiversity functional. Front. Ecol. Environ. 6, 430–438. https://doi.org/10.1890/070081

Letourneau, D.K., Goldstein, B., 2001. Pest damage and arthropod community structure in organic vs. conventional tomato production in California. J. Appl. Ecol. 38, 557–570. https://doi.org/10.1046/j.1365-2664.2001.00611.x

Letourneau, D.K., Jedlicka, J. a., Bothwell, S.G., Moreno, C.R., 2009. Effects of Natural Enemy Biodiversity on the Suppression of Arthropod Herbivores in Terrestrial Ecosystems. Annu. Rev. Ecol. Evol. Syst. 40, 573–592.

https://doi.org/10.1146/annurev.ecolsys.110308.120320

Letourneau, D.K., Drinkwater, L.E.E., Shennan, C., 1996. Effects of soil management on crop nitrogen and insect damage in organic vs. conventional tomato fields. Agric. Ecosyst. Environ. 57, 179–187. https://doi.org/10.1016/0167-8809(96)01027-4

Lewis, W.J., Lenteren, J.C. Van, Phatak, S.C., Tumlinson, J.H., 1997. A total system approach to sustainable pest management. Proc. Natl. Acad. Sci. U. S. A. 94, 12243–12248.

Lobley, M., Butler, A., Reed, M., 2009. The contribution of organic farming to rural development: An exploration of the socio-economic linkages of organic and non-organic farms in England. Land use policy 26, 723–735. https://doi.org/10.1016/j.landusepol.2008.09.007

Lohaus, K., Vidal, S., Thies, C., 2013. Farming practices change food web structures in cereal aphid-parasitoid-hyperparasitoid communities. Oecologia 171, 249–259. https://doi.org/10.1007/s00442-012-2387-8

Loker, A., Francis, C., 2020. Urban food sovereignty: urgent need for agroecology and systems thinking in a post-COVID-19 future. Agroecol. Sustain. Food Syst. https://doi.org/10.1080/21683565.2020.1775752

Long, A., Heitman, J., Tobias, C., Philips, R., Song, B., 2013. Co-occurring anammox, denitrification, and codenitrification in agricultural soils. Appl. Environ. Microbiol. 79, 168–76. https://doi.org/10.1128/AEM.02520-12

Lopes Soares, W., Firpo de Souza Porto, M., 2009. Estimating the social cost of pesticide use: An assessment from acute poisoning in Brazil. Ecol. Econ. 68, 2721–2728. https://doi.org/10.1016/j.ecolecon.2009.05.008

Losey, J.E., Vaughan, M., 2006. The Economic Value of Ecological Services Provided by Insects. Bioscience 56, 311. https://doi.org/10.1641/0006-3568(2006)56[311:tevoes]2.0.co;2

Luck, R.F., Shepard, B.M., Kenmore, P.E., 1988. Experimental methods for evaluating arthropod natural enemies. Annu. Rev. Entomol. Vol. 33 367–391. https://doi.org/10.1146/annurev.ento.33.1.367

Luff, M.L., 1975. Some Features Influencing the Efficiency of Pitfall Traps. Oecologia 19, 345–357.

Lundgren, J.G., Fergen, J.K., 2011. Enhancing predation of a subterranean insect pest: A conservation benefit of winter vegetation in agroecosystems. Appl. Soil Ecol. 51, 9–16. https://doi.org/10.1016/j.apsoil.2011.08.005

Lundgren, J.G., Shaw, J.T., Zaborski, E.R., Eastman, C.E., 2006. The influence of organic transition systems on beneficial ground-dwelling arthropods and predation of insects and weed seeds. Renew. Agric. Food Syst. 21, 227–237. https://doi.org/10.1079/raf2006152

Lundgren, J.G., Toepfer, S., Haye, T., Kuhlmann, U., 2010. Haemolymph defence of an

invasive herbivore: Its breadth of effectiveness against predators. J. Appl. Entomol. 134, 439–448. https://doi.org/10.1111/j.1439-0418.2009.01478.x

Macfadyen, S., Gibson, R., Polaszek, A., Morris, R.J., Craze, P.G., Planqué, R., Symondson, W.O.C., Memmott, J., 2009. Do differences in food web structure between organic and conventional farms affect the ecosystem service of pest control? Ecol. Lett. 12, 229–38. https://doi.org/10.1111/j.1461-0248.2008.01279.x

MacMillan, T., Benton, T., 2014. Engage farmers in research. Nature 509, 25–27. https://doi.org/10.1038/509025a

Mäder, P., Fließbach, A., Dubois, D., Gunst, L., Fried, P., Niggli, U., 2002. Soil fertility and biodiversity in organic farming. Science (80-.). 296, 1694–1697. https://doi.org/10.1126/science.1071148

Magdoff, F., 2007. Ecological agriculture: Principles, practices, and constraints. Renew. Agric. Food Syst. 22, 109–117. https://doi.org/10.1017/S1742170507001846

Mander, Ü., Mikk, M., Külvik, M., 1999. Ecological and low intensity agriculture as contributors to landscape and biological diversity. Landsc. Urban Plan. 46, 169–177. https://doi.org/10.1016/S0169-2046(99)00042-0

Marja, R., Herzon, I., Viik, E., Elts, J., Mänd, M., Tscharntke, T., Batáry, P., 2014. Environmentally friendly management as an intermediate strategy between organic and conventional agriculture to support biodiversity. Biol. Conserv. 178, 146–154. https://doi.org/10.1016/j.biocon.2014.08.005

Maroni, M., Fanetti, A.C., Metruccio, F., 2006. Risk assessment and management of occupational exposure to pesticides in agriculture, in: Medicina Del Lavoro. Mattioli, pp. 430–437.

Martínez-Sastre, R., García, D., Miñarro, M., Martín-López, B., 2020. Farmers' perceptions and knowledge of natural enemies as providers of biological control in cider apple orchards. J. Environ. Manage. 266. https://doi.org/10.1016/j.jenvman.2020.110589

McHugh, N.M., Moreby, S., Lof, M.E., Van der Werf, W., Holland, J.M., 2020. The contribution of semi-natural habitats to biological control is dependent on sentinel prey type. J. Appl. Ecol. 914–925. https://doi.org/10.1111/1365-2664.13596

Melbourne, B.A., 1999. Bias in the effect of habitat structure on pitfall traps: An experimental evaluation. Austral Ecol. https://doi.org/10.1046/j.1442-9993.1999.00967.x

Mesmin, X., Cortesero, A.M., Daniel, L., Plantegenest, M., Faloya, V., Le Ralec, A., 2020. Influence of soil tillage on natural regulation of the cabbage root fly Delia radicum in brassicaceous crops. Agric. Ecosyst. Environ. 293, 106834. https://doi.org/10.1016/j.agee.2020.106834

Messelink, G.J., Slooten, M. Van, 2004. Effects of soil-dwelling predators and organic treatments on the cabbage root fly Delia radicum (Diptera : Anthomyiidae) in greenhouse radish. Proc. Netherlands Entomol. Soc. 15, 87–91.

Meyer, G.A., 2000. Interactive effects of soil fertility and herbivory on Brassica nigra. Oikos 88, 433–441. https://doi.org/10.1034/j.1600-0706.2000.880221.x

Meyling, N.V., 2007. Methods for isolation of entomopathogenic fungi from the soil environment, Deliverable 5.1, VegQure, DARCOF III: Research in Organic Food and Farming (FØJO III).

Meyling, N. V., Eilenberg, J., 2007. Ecology of the entomopathogenic fungi Beauveria bassiana and Metarhizium anisopliae in temperate agroecosystems: Potential for conservation biological control. Biol. Control 43, 145–155. https://doi.org/10.1016/j.biocontrol.2007.07.007

Meyling, N. V., Eilenberg, J., 2006. Occurrence and distribution of soil borne entomopathogenic fungi within a single organic agroecosystem. Agric. Ecosyst. Environ. 113, 336–341. https://doi.org/10.1016/j.agee.2005.10.011

Meyling, N. V., Navntoft, S., Philipsen, H., Thorup-Kristensen, K., Eilenberg, J., 2013. Natural regulation of Delia radicum in organic cabbage production. Agric. Ecosyst. Environ. 164, 183–189. https://doi.org/10.1016/j.agee.2012.09.019

Meyling, N. V., Thorup-Kristensen, K., Eilenberg, J., 2011. Below- and aboveground abundance and distribution of fungal entomopathogens in experimental conventional and organic cropping systems. Biol. Control 59, 180–186. https://doi.org/10.1016/j.biocontrol.2011.07.017

Michereff-Filho, M., Guedes, R.N.C., Della-Lucia, T.M.C., Michereff, M.F.F., Cruz, I., 2004. Non-target impact of chlorpyrifos on soil arthropods associated with no-tillage cornfields in Brazil. Int. J. Pest Manag. 50, 91–99. https://doi.org/10.1080/09670870410001655885

Mietkiewski, R.T., Pell, J.K., Clark, S.J., 1997. Influence of pesticide use on the natural occurrence of entomopathogenic fungi in arable soils in the UK: Field and laboratory comparisons. Biocontrol Sci. Technol. 7, 565–576. https://doi.org/10.1080/09583159730622

Mitchell, B., 1963. Ecology of Two Carabid Beetles, Bembidion lampros (Herbst) and Trechus quadristriatus (Schrank). J. Anim. Ecol. 32, 289. https://doi.org/10.2307/2542

Mkenda, P.A., Ndakidemi, P.A., Stevenson, P.C., Arnold, S.E.J., Darbyshire, I., Belmain, S.R., Priebe, J., Johnson, A.C., Tumbo, J., Gurr, G.M., 2020. Knowledge gaps among smallholder farmers hinder adoption of conservation biological control. Biocontrol Sci. Technol. 30, 256–277. https://doi.org/10.1080/09583157.2019.1707169

Monokrousos, N., Papatheodorou, E.M., Diamantopoulos, J.D., Stamou, G.P., 2006. Soil quality variables in organically and conventionally cultivated field sites. Soil Biol. Biochem. 38, 1282–1289. https://doi.org/10.1016/j.soilbio.2005.09.023

Montgomery, D.R., 2007. Soil erosion and agricultural sustainability. Proc. Natl. Acad. Sci. U. S. A. 104, 13268–13272. https://doi.org/10.1073/pnas.0611508104

Moore, J.C., 1994. Impact of agricultural practices on soil food web structure: Theory and

application. Agric. Ecosyst. Environ. 51, 239–247. https://doi.org/10.1016/0167-8809(94)90047-7

Mowat, D.J., Coaker, T.H., 1967. The toxicity of some soil insecticides to carabid predators of the cabbage root fly (Erioischia brassicae (Bouché)). Ann. Appl. Biol. 59, 349–354. https://doi.org/10.1111/j.1744-7348.1967.tb04451.x

Muilenburg, V.L., Goggin, F.L., Hebert, S.L., Jia, L., Stephen, F.M., 2008. Ant predation on red oak borer confirmed by field observation and molecular gut-content analysis. Agric. For. Entomol. 10, 205–213. https://doi.org/10.1111/j.1461-9563.2008.00371.x

Mukerji, M.K., 1971. Major factors in survival of the immature stages of hylemya brasszcae (diptera: anthomyiidae) on cabbage. Can. Entomol. 103, 717–728. https://doi.org/10.4039/Ent103717-5

Mulder, C., Helder, J., Vervoort, M.T.W.W., Arie Vonk, J., 2011. Trait-mediated diversification in nematode predator-prey systems. Ecol. Evol. 1, 386–391. https://doi.org/10.1002/ece3.36

Murtaugh, P.A., 2014. In defense of *P* values. Ecology 95, 611–617. https://doi.org/10.1890/13-0590.1

Myrand, V., Buffet, J.P., Guertin, C., 2015. Susceptibility of Cabbage Maggot Larvae (Diptera: Anthomyiidae) to Hypocreales Entomopathogenic Fungi. J. Econ. Entomol. 108, 34–44. https://doi.org/10.1093/jee/tou019

Naranjo, S.E., Ellsworth, P.C., Frisvold, G.B., 2015. Economic Value of Biological Control in Integrated Pest Management of Managed Plant Systems. Annu. Rev. Entomol. 60, 621–645. https://doi.org/10.1146/annurev-ento-010814-021005

Neveu, N., Grandgirard, J., Nenon, J.P., Cortesero, a M., 2002. Systemic release of herbivore-induced plant volatiles by turnips infested by concealed root-feeding larvae Delia radicum L. J. Chem. Ecol. 28, 1717–32.

Neveu, N., Krespi, L., Kacem, N., Nenon, J.-P., 2000. Host-stage selection by Trybliographa rapae, a parasitoid of the cabbage root fly Delia radicum. Entomol. Exp. Appl. 96, 231–237. https://doi.org/10.1046/j.1570-7458.2000.00701.x

Nielsen, O., Philipsen, H., 2004. Occurrence of Steinernema species in cabbage fields and the effect of inoculated S. feltiae on Delia radicum and its parasitoids. Agric. For. Entomol. 6, 25–30. https://doi.org/10.1111/j.1461-9555.2004.00198.x

Nielsen, U.N., Ayres, E., Wall, D.H., Bardgett, R.D., 2011. Soil biodiversity and carbon cycling: a review and synthesis of studies examining diversity-function relationships. Eur. J. Soil Sci. 62, 105–116. https://doi.org/10.1111/j.1365-2389.2010.01314.x

Nilsson, U., 2011. Conservation Biological Control of Insect Pests in Two Horticultural Crops.

Nilsson, U., Rännbäck, L.-M., Anderson, P., Rämert, B., 2012. Herbivore response to habitat manipulation with floral resources: A study of the cabbage root fly. J. Appl. Entomol. 136, 481–489. https://doi.org/10.1111/j.1439-0418.2011.01685.x

Nilsson, U., Rännbäck, L.-M.M., Anderson, P., Björkman, M., Futter, M., Rämert, B., 2016. Effects of conservation strip and crop type on natural enemies of Delia radicum. J. Appl. Entomol. 140, 287–298. https://doi.org/10.1111/jen.12256

Noble, D.W.A., Lagisz, M., O'dea, R.E., Nakagawa, S., 2017. Nonindependence and sensitivity analyses in ecological and evolutionary meta-analyses. Mol. Ecol. 26, 2410–2425. https://doi.org/10.1111/mec.14031

Nottingham, S.F., 1988. Host-plant finding for oviposition by adult cabbage root fly, Delia radicum. J. Insect Physiol. 34, 227–234. https://doi.org/10.1016/0022-1910(88)90053-4

Onstad, D.W., Knolhoff, L.M., 2009. Finding the economics in economic entomology. J. Econ. Entomol. 102, 1–7. https://doi.org/10.1603/ice.2016.93311

Opdam, P., Coninx, I., Dewulf, A., Steingröver, E., Vos, C., van der Wal, M., 2016. Does information on landscape benefits influence collective action in landscape governance? Curr. Opin. Environ. Sustain. https://doi.org/10.1016/j.cosust.2015.12.006

Orgiazzi, A., Bardgett, R.D., Barrios, E., Behan-Pelletier, V., Briones, M.J.I., Chotte, J.-L., De Deyn, G.B., Eggleton, P., Fierer, N., Fraser, T., Hedlund, K., Jeffery, S., Johnson, N.C., Jones, A., Kandeler, E., Kaneko, N., Lavelle, P., Lemanceau, P., Miko, L., Montanarella, L., Moreira, F.M.S., Ramirez, K.S., Scheu, S., Singh, B.K., Six, J., van der Putten, W.H., Wall, D.H. (Eds.), 2016. Global Soil Biodiversity Atlas, European Commission Joint Research CentreJRC.

Orr, C.H., James, A., Leifert, C., Cooper, J.M., Cummings, S.P., 2011. Diversity and activity of free-living nitrogen-fixing bacteria and total bacteria in organic and conventionally managed soils. Appl. Environ. Microbiol. 77, 911–9. https://doi.org/10.1128/AEM.01250-10

Orr, C.H., Leifert, C., Cummings, S.P., Cooper, J.M., 2012. Impacts of organic and conventional crop management on diversity and activity of free-living nitrogen fixing bacteria and total bacteria are subsidiary to temporal effects. PLoS One 7, e52891. https://doi.org/10.1371/journal.pone.0052891

Ourry, M., Lebreton, L., Chaminade, V., Guillerm-Erckelboudt, A.Y., Hervé, M., Linglin, J., Marnet, N., Ourry, A., Paty, C., Poinsot, D., Cortesero, A.M., Mougel, C., 2018. Influence of belowground herbivory on the dynamics of root and rhizosphere microbial communities. Front. Ecol. Evol. 6, 91. https://doi.org/10.3389/fevo.2018.00091

Palmer, M.W., Cooper, J., Tétard-jones, C., Srednicka-tober, D., Eyre, M., Shotton, P.N., Volakakis, N., Cakmak, I., Ozturk, L., Leifert, C., Wilcockson, S.J., Bilsborrow, P.E., 2013. The influence of organic and conventional fertilisation and crop protection practices , preceding crop , harvest year and weather conditions on yield and quality of potato (Solanum tuberosum) in a long-term management trial. Eur. J. Agron. 49, 83–92.

Pandey, S., Singh, D.K., 2004. Total bacterial and fungal population after chlorpyrifos and quinalphos treatments in groundnut (Arachis hypogaea L.) soil. Chemosphere 55, 197–

205. https://doi.org/10.1016/j.chemosphere.2003.10.014

Papadopoulou, G. V., Dam, N.M. van, 2016. Mechanisms and ecological implications of plant-mediated interactions between belowground and aboveground insect herbivores. Ecol. Res. 32, 13–26. https://doi.org/10.1007/S11284-016-1410-7

Pell, J.K., Hannam, J.J., Steinkraus, D.C., 2010. Conservation biological control using fungal entomopathogens. BioControl 55, 187–198. https://doi.org/10.1007/s10526-009-9245-6

Perović, D.J., Gámez-Virués, S., Landis, D.A., Wäckers, F., Gurr, G.M., Wratten, S.D., You, M.-S., Desneux, N., 2017. Managing biological control services through multi-trophic trait interactions: review and guidelines for implementation at local and landscape scales. Biol. Rev. https://doi.org/10.1111/brv.12346

Petersen, B., Snapp, S., 2015. What is sustainable intensification? Views from experts. Land use policy 46, 1–10. https://doi.org/10.1016/j.landusepol.2015.02.002

Petit, C., Aubry, C., 2016. Typology of organic management styles in a cash-crop region using a multi-criteria method. Org. Agric. 6, 155–169. https://doi.org/10.1007/s13165-015-0124-4

Pfiffner, L., Luka, H., 2003. Effects of low-input farming systems on carabids and epigeal spiders – a paired farm approach. Basic Appl. Ecol. 4, 117–127. https://doi.org/10.1078/1439-1791-00121

Phelan, P.L., Norris, K.H., Mason, J.F., 1996. Soil-Management History and Host Preference by Ostrinia nubilalis: Evidence for Plant Mineral Balance Mediating Insect-Plant Interactions. Environ. Entomol. 25, 1329–1336. https://doi.org/10.1093/ee/25.6.1329

Pierre, P.S., Dugravot, S., Cortesero, A.-M., Poinsot, D., Raaijmakers, C.E., Hassan, H.M., van Dam, N.M., 2012. Broccoli and turnip plants display contrasting responses to belowground induction by Delia radicum infestation and phytohormone applications. Phytochemistry 73, 42–50. https://doi.org/10.1016/j.phytochem.2011.09.009

Pierre, P.S., Dugravot, S., Ferry, A., Soler, R., Van Dam, N.M., Cortesero, A.., 2011. Aboveground herbivory affects indirect defences of brassicaceous plants against the root feeder Delia radicum Linnaeus: laboratory and field evidence. Ecol. Entomol. 36, 326–334. https://doi.org/10.1111/j.1365-2311.2011.01276.x

Pieterse, C.M.J., Zamioudis, C., Berendsen, R.L., Weller, D.M., Van Wees, S.C.M., Bakker, P.A.H.M., 2014. Induced Systemic Resistance by Beneficial Microbes. Annu. Rev. Phytopathol. 52, 347–375. https://doi.org/10.1146/annurev-phyto-082712-102340

Pimentel, D., 2005. Environmental and economic costs of the application of pesticides primarily in the United States. Environ. Dev. Sustain. 7, 229–252. https://doi.org/10.1007/s10668-005-7314-2

Pimentel, D., Hepperly, P., Hanson, J., Siede, R., Douds, D., 2005. Organic and conventional farming systems: Environmental and economic issues. Tech. Report. New York State Coll. Agric. Life Sci. Cornell Univ. Ithaca, N.Y.

(http//ecommons.library.cornell.edu/bitstream/1813/2101/1/pimentel_report_05-1.pdf). 5, 1–52.

Pimentel, D., Peshin, R., 2014. Integrated pest management , Integrated Pest Management: Pesticide Problems, Vol.3. Springer Netherlands. https://doi.org/10.1007/978-94-007-7796-5_1

Pineda, A., Zheng, S.J., van Loon, J.J.A., Pieterse, C.M.J., Dicke, M., 2010. Helping plants to deal with insects: The role of beneficial soil-borne microbes. Trends Plant Sci. 15, 507–514. https://doi.org/10.1016/j.tplants.2010.05.007

Pissonnier, S., Dufils, A., Le Gal, P.-Y., 2019. A methodology for redesigning agroecological radical production systems at the farm level. Agric. Syst. 173, 161–171. https://doi.org/10.1016/J.AGSY.2019.02.018

Ponisio, L.C., M'gonigle, L.K., Mace, K.C., Palomino, J., Valpine, P. De, Kremen, C., 2015. Diversification practices reduce organic to conventional yield gap. Proc. R. Soc. B Biol. Sci. 282. https://doi.org/10.1098/rspb.2014.1396

Poveda, K., Steffan-Dewenter, I., Scheu, S., Tscharntke, T., 2006. Belowground effects of organic and conventional farming on aboveground plant–herbivore and plant–pathogen interactions. Agric. Ecosyst. Environ. 113, 162–167. https://doi.org/10.1016/j.agee.2005.09.005

Prager, K., 2015. Agri-environmental collaboratives for landscape management in Europe. Curr. Opin. Environ. Sustain. https://doi.org/10.1016/j.cosust.2014.10.009

Prager, K., Reed, M., Scott, A., 2012. Encouraging collaboration for the provision of ecosystem services at a landscape scale-Rethinking agri-environmental payments. Land use policy 29, 244–249. https://doi.org/10.1016/j.landusepol.2011.06.012

Prasad, R.P., Snyder, W.E., 2006. Polyphagy complicates conservation biological control that targets generalist predators. J. Appl. Ecol. 43, 343–352. https://doi.org/10.1111/j.1365-2664.2006.01129.x

Prasad, R.P., Snyder, W.E., 2004. Predator interference limits fly egg biological control by a guild of ground-active beetles. Biol. Control 31, 428–437. https://doi.org/10.1016/j.biocontrol.2004.07.005

Prasifka, J.R., Lopez, M.D., Hellmich, R.L., Lewis, L.C., Dively, G.P., 2007. Comparison of pitfall traps and litter bags for sampling ground-dwelling arthropods. J. Appl. Entomol. 131, 115–120. https://doi.org/10.1111/j.1439-0418.2006.01141.x

Pretty, J., 2018. Intensification for redesigned and sustainable agricultural systems. Science (80-.). 908, 1–7. https://doi.org/10.1126/science.aav0294

Pretty, J., Benton, T.G., Bharucha, Z.P., Dicks, L. V., Flora, C.B., Godfray, H.C.J., Goulson, D., Hartley, S., Lampkin, N., Morris, C., Pierzynski, G., Prasad, P.V.V., Reganold, J., Rockström, J., Smith, P., Thorne, P., Wratten, S., 2018. Global assessment of agricultural system redesign for sustainable intensification. Nat. Sustain. 1, 441–446. https://doi.org/10.1038/s41893-018-0114-0

Pretty, J., Bharucha, Z.P., 2015. Integrated pest management for sustainable intensification of agriculture in Asia and Africa. Insects 6, 152–182. https://doi.org/10.3390/insects6010152

Pretty, J., Bharucha, Z.P., 2014. Sustainable intensification in agricultural systems. Ann. Bot. 114, 1571–1596. https://doi.org/10.1093/aob/mcu205

Pretty, J.N., Brett, C., Gee, D., Hine, R.E., Mason, C.F., 2000. An assessment of the total external costs of UK agriculture 65.

Puech, C., Baudry, J., Joannon, A., Poggi, S., Aviron, S., 2014. Organic vs. conventional farming dichotomy: Does it make sense for natural enemies? Agric. Ecosyst. Environ. 194, 48–57. https://doi.org/10.1016/j.agee.2014.05.002

Purtauf, T., Roschewitz, I., Dauber, J., Thies, C., Tscharntke, T., Wolters, V., 2005. Landscape context of organic and conventional farms: Influences on carabid beetle diversity. Agric. Ecosyst. Environ. 108, 165–174. https://doi.org/10.1016/j.agee.2005.01.005

Pywell, R.F., Heard, M.S., Woodcock, B.A., Hinsley, S., Ridding, L., Nowakowski, M., Bullock, J.M., 2015. Wildlife-friendly farming increases crop yield: Evidence for ecological intensification. Proc. R. Soc. B Biol. Sci. 282. https://doi.org/10.1098/rspb.2015.1740

Quesada-Moraga, E., Navas-Cortés, J.A., Maranhao, E.A.A., Ortiz-Urquiza, A., Santiago-Álvarez, C., 2007. Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. Mycol. Res. 111, 947–966. https://doi.org/10.1016/j.mycres.2007.06.006

Ramarao, N., Nielsen-Leroux, C., Lereclus, D., 2012. The insect Galleria mellonella as a powerful infection model to investigate bacterial pathogenesis. J. Vis. Exp. 1–7. https://doi.org/10.3791/4392

Ramos, Y., Portal, O., Lysøe, E., Meyling, N. V., Klingen, I., 2017. Diversity and abundance of Beauveria bassiana in soils, stink bugs and plant tissues of common bean from organic and conventional fields. J. Invertebr. Pathol. 150, 114–120. https://doi.org/10.1016/j.jip.2017.10.003

Rand, T.A., Tylianakis, J.M., Tscharntke, T., 2006. Spillover edge effects: The dispersal of agriculturally subsidized insect natural enemies into adjacent natural habitats. Ecol. Lett. https://doi.org/10.1111/j.1461-0248.2006.00911.x

Rännbäck, L.-M., 2015. Biological control strategies against the cabbage root fly Delia radicum.

Rännbäck, L.-M., Cotes, B., Anderson, P., Rämert, B., Meyling, N. V., 2015. Mortality risk from entomopathogenic fungi affects oviposition behavior in the parasitoid wasp Trybliographa rapae. J. Invertebr. Pathol. 124, 78–86. https://doi.org/10.1016/j.jip.2014.11.003

Ratnadass, A., Michellon, R., Randriamanantsoa, R., Séguy, L., 2006. Effects of Soil and Plant Managementon Crop Pests and Diseases 589–602. https://doi.org/10.1201/9781420017113.ch41

Razinger, J., Lutz, M., Grunder, J., Urek, G., 2018. Laboratory Investigation of Cauliflower–Fungus–Insect Interactions for Cabbage Maggot Control. J. Econ. Entomol. https://doi.org/10.1093/jee/toy228

Razinger, J., Lutz, M., Schroers, H.-J., Palmisano, M., Wohler, C., Urek, G., Grunder, J., 2014. Direct plantlet inoculation with soil or insect-associated fungi may control cabbage root fly maggots. J. Invertebr. Pathol. 120, 59–66. https://doi.org/10.1016/j.jip.2014.05.006

Razinger, J., Žerjav, M., Zemljič-Urbančič, M., Modic, Š., Lutz, M., Schroers, H.-J., Grunder, J., Fellous, S., Urek, G., 2017. Comparison of cauliflower-insect-fungus interactions and pesticides for cabbage root fly control. Insect Sci. https://doi.org/10.1111/1744-7917.12534

Read, D.C., 1962. Notes on the Life History of Aleochara bilineata (Gyll.) (Coleoptera: Staphylinidae), and on Its Potential Value as a Control Agent for the Cabbage Maggot, Hylemya brassicae (Bouch) (Diptera: Anthomyiidae). Can. Entomol. 94, 417–424. https://doi.org/10.4039/Ent94417-4

Rebek, E.J., Sadof, C.S., Hanks, L.M., 2005. Manipulating the abundance of natural enemies in ornamental landscapes with floral resource plants. Biol. Control 33, 203–216. https://doi.org/10.1016/j.biocontrol.2005.02.011

Reeves, T.G., Thomas, G., Ramsay, G., 2016. Save and grow in practice: maize, rice, wheat, Journal of Chemical Information and Modeling. https://doi.org/10.1017/CBO9781107415324.004

Reilly, K., Cullen, E., Lola-Luz, T., Stone, D., Valverde, J., Gaffney, M., Brunton, N., Grant, J., Griffiths, B.S., 2013. Effect of organic, conventional and mixed cultivation practices on soil microbial community structure and nematode abundance in a cultivated onion crop. J. Sci. Food Agric. https://doi.org/10.1002/jsfa.6206

Richardson, P., Long, S., Hart, A., Willmott, D., Chandler, D., 2002. Susceptibility of cabbage root fly Delia radicum, in potted cauliflower (Brassica oleracea var. botrytis) to isolates of entomopathogenic nematodes (Steinernema and Heterorhabditis spp.) indigenous to the UK. Nematology 4, 965–970. https://doi.org/10.1163/156854102321122584

Riley, H., Pommeresche, R., Eltun, R., Hansen, S., Korsaeth, A., 2008. Soil structure, organic matter and earthworm activity in a comparison of cropping systems with contrasting tillage, rotations, fertilizer levels and manure use. Agric. Ecosyst. Environ. 124, 275–284. https://doi.org/10.1016/j.agee.2007.11.002

Robinson, D.A., Panagos, P., Borrelli, P., Jones, A., Montanarella, L., Tye, A., Obst, C.G., 2017. Soil natural capital in Europe; A framework for state and change assessment. Sci. Rep. 7, 1–14. https://doi.org/10.1038/s41598-017-06819-3

Rockström, J., Williams, J., Daily, G., Noble, A., Matthews, N., Gordon, L., Wetterstrand, H., DeClerck, F., Shah, M., Steduto, P., de Fraiture, C., Hatibu, N., Unver, O., Bird, J., Sibanda, L., Smith, J., 2017. Sustainable intensification of agriculture for human prosperity and global sustainability. Ambio 46, 4–17. https://doi.org/10.1007/s13280-016-0793-6

Roessingh, P., Stadler, E., Baur, R., 1997. Tarsal chemoreceptors and oviposition behaviour of the cabbage root fly (Delia radicum) sensitive to fractions and new compounds of host-leaf surface extracts 140–148.

Roger-Estrade, J., Anger, C., Bertrand, M., Richard, G., 2010. Tillage and soil ecology: Partners for sustainable agriculture. Soil Tillage Res. 111, 33–40. https://doi.org/10.1016/j.still.2010.08.010

Romaní, A.M., Fischer, H., Mille-Lindblom, C., Tranvik, L.J., 2006. Interactions of bacteria and fungi on decomposing litter: Differential extracellular enzyme activities. Ecology 87, 2559–2569. https://doi.org/10.1890/0012-9658(2006)87[2559:IOBAFO]2.0.CO;2

Rosenheim, J.A., 1998. Higher-Order Predators and the Regulation of Insect Herbivore Populations. Annu. Rev. Entomol. 43, 421–447. https://doi.org/10.1146/annurev.ento.43.1.421

Roubinet, E., Birkhofer, K., Malsher, G., Staudacher, K., Ekbom, B., Traugott, M., Jonsson, M., 2017. Diet of generalist predators reflects effects of cropping period and farming system on extra- and intraguild prey. Ecol. Appl. https://doi.org/10.1002/eap.1510

Rousse, P., Fournet, S., Porteneuve, C., Brunel, E., 2003. Trap cropping to control Delia radicum populations in cruciferous crops: First results and future applications. Entomol. Exp. Appl. 109, 133–138. https://doi.org/10.1046/j.1570-7458.2003.00098.x

Ruiu, L., Satta, A., Floris, I., 2013. Emerging entomopathogenic bacteria for insect pest management. Bull. Insectology 66, 181–186.

Rusch, A., Bommarco, R., Ekbom, B., 2017. Conservation Biological Control in Agricultural Landscapes. Adv. Bot. Res. 81, 333–360. https://doi.org/10.1016/bs.abr.2016.11.001

Rusch, A., Chaplin-Kramer, R., Gardiner, M.M., Hawro, V., Holland, J., Landis, D., Thies, C., Tscharntke, T., Weisser, W.W., Winqvist, C., Woltz, M., Bommarco, R., 2016. Agricultural landscape simplification reduces natural pest control: A quantitative synthesis. Agric. Ecosyst. Environ. 221, 198–204. https://doi.org/10.1016/j.agee.2016.01.039

Rusch, A., Valantin-Morison, M., Sarthou, J.P., Roger-Estrade, J., 2010. Biological control of insect pests in agroecosystems. Effects of crop management, farming systems, and seminatural habitats at the landscape scale: A review, Advances in Agronomy. Academic Press. https://doi.org/10.1016/B978-0-12-385040-9.00006-2

Ruttan, V.W., 2015. The Transition to Agricultural Sustainability. Can Econ. Growth Be Sustain. Collect. Pap. Vernon W. Ruttan Yujiro Hayami 96, 5960–5967. https://doi.org/10.1093/acprof:osobl/9780199754359.003.0013

Ryan, J., Ryan, M.F., 1980. Observations on the natural mortality of the overwintering pupa of the cabbage root fly, Delia brassicae (Wiedemann), in Ireland. Plant Pathol. 29, 38–44. https://doi.org/10.1111/j.1365-3059.1980.tb01135.x

Rypstra, A.L., Marshall, S.D., 2005. Augmentation of soil detritus affects the spider community and herbivory in a soybean agroecosystem. Entomol. Exp. Appl. 116, 149–

157. https://doi.org/10.1111/j.1570-7458.2005.00322.x

Salter, D.D., Hughes, R.. D., 1959. Natural Mortality of Erioischia brassicae (Bouche) (Diptera , Anthomyiidae) During the Immature Stages of the First Generation. J. Anim. Ecol. 28, 231–241. https://doi.org/10.2307/2080

Sánchez-Bayo, F., Wyckhuys, K.A.G., 2019. Worldwide decline of the entomofauna: A review of its drivers. Biol. Conserv. 232, 8–27. https://doi.org/10.1016/j.biocon.2019.01.020

Sánchez-Moreno, S., Nicola, N.L., Ferris, H., Zalom, F.G., 2009. Effects of agricultural management on nematode–mite assemblages: Soil food web indices as predictors of mite community composition. Appl. Soil Ecol. 41, 107–117. https://doi.org/10.1016/j.apsoil.2008.09.004

Scheu, S., 2002. The soil food web: structure and perspectives. Eur. J. Soil Biol. 38, 11–20. https://doi.org/10.1016/S1164-5563(01)01117-7

Schrama, M., de Haan, J.J., Kroonen, M., Verstegen, H., Van der Putten, W.H., 2018. Crop yield gap and stability in organic and conventional farming systems. Agric. Ecosyst. Environ. 256, 123–130. https://doi.org/10.1016/j.agee.2017.12.023

Schulte, R.P.O., Creamer, R.E., Donnellan, T., Farrelly, N., Fealy, R., O'Donoghue, C., O'hUallachain, D., 2014. Functional land management: A framework for managing soil-based ecosystem services for the sustainable intensification of agriculture. Environ. Sci. Policy 38, 45–58. https://doi.org/10.1016/j.envsci.2013.10.002

Scriber, J.M., 1984. Nitrogen Nutrition of Plants and Insect Invasion. Nitrogen Crop Prod. 441–460. https://doi.org/10.2134/1990.nitrogenincropproduction.c29

Scullion, J., Neale, S., Philipps, L., 2002. Comparisons of earthworm populations and cast properties in conventional and organic arable rotations. Soil Use Manag. 18, 293–300. https://doi.org/10.1079/sum2002132

Settele, J., Settle, W.H., 2018. Conservation biological control: Improving the science base. Proc. Natl. Acad. Sci. U. S. A. 115, 8241–8243. https://doi.org/10.1073/pnas.1810334115

Seufert, V., Ramankutty, N., Foley, J.A., 2012. Comparing the yields of organic and conventional agriculture. Nature 485, 229–232. https://doi.org/10.1038/nature11069

Shapiro-Ilan, D.I., Jackson, M., Reilly, C.C., Hotchkiss, M.W., 2004. Effects of combining an entomopathogenic fungi or bacterium with entomopathogenic nematodes on mortality of Curculio caryae (Coleoptera: Curculionidae). Biol. Control 30, 119–126. https://doi.org/10.1016/j.biocontrol.2003.09.014

Shields, M.W., Johnson, A.C., Pandey, S., Cullen, R., González-Chang, M., Wratten, S.D., Gurr, G.M., Chang, M.G.-, González- Chang, M., Wratten, S.D., Gurr, G.M., 2019. History , current situation and challenges for conservation biological control. Biol. Control 131, 25–35. https://doi.org/10.1016/j.biocontrol.2018.12.010

Snyder, W.E., 2019. Give predators a complement: Conserving natural enemy biodiversity to improve biocontrol. Biol. Control 135, 73–82. https://doi.org/10.1016/J.BIOCONTROL.2019.04.017

Snyder, W.E., Ives, A.R., 2003. Interactions between specialist and generalist natural enemies: parasitoids, predators and pea aphid biocontrol. Ecology 84, 91–107. https://doi.org/10.1890/0012-9658(2003)084[0091:IBSAGN]2.0.CO;2

Soler, R., Schaper, S., Bezemer, T.M., Cortesero, A.M., Hoffmeister, T.S., van der Putten, W.H., Vet, L.E.M., Harvey, J.A., 2009. Influence of presence and spatial arrangement of belowground insects on host-plant selection of aboveground insects: a field study. Ecol. Entomol. 34, 339–345. https://doi.org/10.1111/j.1365-2311.2008.01082.x

Sontowski, R., Gorringe, N.J., Pencs, S., Schedl, A., Touw, A.J., van Dam, N.M., 2019. Same difference? Low and high glucosinolate Brassica rapa varieties show similar responses upon feeding by two specialist root herbivores. Front. Plant Sci. 10, 1451. https://doi.org/10.3389/FPLS.2019.01451

Spence, J.R., 1994. Sampling carabid assemblages with pitfall traps: The madness and the method. Can. Entomol. 126, 881–894. https://doi.org/10.4039/Ent126881-3

Stanhill, G., 1990. The comparative productivity of organic agriculture. Agric. Ecosyst. Environ. 30, 1–26. https://doi.org/10.1016/0167-8809(90)90179-H

Stavi, I., Bel, G., Zaady, E., 2016. Soil functions and ecosystem services in conventional, conservation, and integrated agricultural systems. A review. Agron. Sustain. Dev. 36. https://doi.org/10.1007/s13593-016-0368-8

Stelinski, L.L., Willett, D., Rivera, M.J., Ali, J.G., 2019. 'Tuning' communication among four trophic levels of the root biome to facilitate biological control. Biol. Control 131, 49–53. https://doi.org/10.1016/j.biocontrol.2019.01.006

Stenberg, J.A., 2017. A Conceptual Framework for Integrated Pest Management. Trends Plant Sci. 22, 759–769. https://doi.org/10.1016/j.tplants.2017.06.010

Stern, V.M., Smith, R.F., van den Bosch, R., Hagen, K.S., 1959. The integration of chemical and biological control of the spotted alfalfa aphid: The integrated control concept. Hilgardia 29, 81–101. https://doi.org/10.3733/hilg.v29n02p081

Stockdale, E. a., Fortune, S., Cuttle, S.P., 2002. Soil fertility in organic farming systems – fundamentally different? Soil Use Manag. 18, 301–308. https://doi.org/10.1079/SUM2002143

Stockdale, E. a., Watson, C. a., 2009. Biological indicators of soil quality in organic farming systems. Renew. Agric. Food Syst. 24, 308. https://doi.org/10.1017/S1742170509990172

Stolze, M., Piorr, A., Haring, A., Dabbert, S., 2000. The Environmental impacts of organic farming in europe.

Straub, C.S., Finke, D.L., Snyder, W.E., 2008. Are the conservation of natural enemy

biodiversity and biological control compatible goals? Biol. Control 45, 225–237. https://doi.org/10.1016/j.biocontrol.2007.05.013

Straub, R.W., 1988. Suppression of Cabbage Root Maggot (Diptera : Anthomyiidae) Damage to Cruciferous Transplants by Incorporation of Granular Insecticide into Potting Soil. J. Econ. Entomol. 81, 578–581. https://doi.org/10.1093/jee/81.2.578

Stuart, R J, El-Borai, F.E., Duncan, L.W., 2008. From Augmentation to Conservation of Entomopathogenic Nematodes: Trophic Cascades, Habitat Manipulation and Enhanced Biological Control of Diaprepes abbreviatus Root Weevils in Florida Citrus Groves. J. Nematol. 40, 73–84.

Stuart, R. J., El-Borai, F.E., Duncan, L.W., El Borai, F.E., Duncan, L.W., 2008. From augmentation to conservation of entomopathogenic nematodes: Trophic cascades, habitat manipulation and enhanced biological control of Diaprepes abbreviatus root weevils in Florida citrus groves. J. Nematol. 40, 73–84.

Sturz, A., Christie, B.., 2003. Beneficial microbial allelopathies in the root zone: the management of soil quality and plant disease with rhizobacteria. Soil Tillage Res. 72, 107–123.

Sutherland, L.A., Gabriel, D., Hathaway-Jenkins, L., Pascual, U., Schmutz, U., Rigby, D., Godwin, R., Sait, S.M., Sakrabani, R., Kunin, W.E., Benton, T.G., Stagl, S., 2012. The "Neighbourhood Effect": A multidisciplinary assessment of the case for farmer co-ordination in agri-environmental programmes. Land use policy 29, 502–512. https://doi.org/10.1016/j.landusepol.2011.09.003

Symondson, W.O., Cesarini, S., Dodd, P.W., Harper, G.L., Bruford, M.W., Glen, D.M., Wiltshire, C.W., Harwood, J.D., 2006. Biodiversity vs . biocontrol : positive and negative effects of alternative prey on control of slugs by carabid beetles. Bull. Entomol. Res. 44, 637–645. https://doi.org/10.1079/BER2006467

Tamburini, G., De Simone, S., Sigura, M., Boscutti, F., Marini, L., 2016. Conservation tillage mitigates the negative effect of landscape simplification on biological control. J. Appl. Ecol. 53, 233–241. https://doi.org/10.1111/1365-2664.12544

The Soil Association, 2019a. Soil Association Standards Farming and growing.

The Soil Association, 2019b. Green Brexit - setting the bar for a green Brexit in Food and Farming.

Thoden, T.C., Korthals, G.W., Termorshuizen, A.J., 2011. Organic amendments and their influences on plant-parasitic and free-living nematodes: A promising method for nematode management? Nematology 13, 133–153. https://doi.org/10.1163/138855410X541834

Thomas, J.., Telfer, G.M., Roy, D.B., Preston, C.D., Greenwood, J.J.D., Asher, J., Fox, R., Clarke, R.T., Lawton, J.H., 2004. Comparative losses of British Butterflies, Birds, and Plants and the Global Extinction crisis. Science (80-.). 303, 1879–1881. https://doi.org/10.1126/science.1095046

Thorbek, P., Bilde, T., 2004. Reduced numbers of generalist arthropod predators after crop management. J. Appl. Ecol. 41, 526–538. https://doi.org/10.1111/j.0021-8901.2004.00913.x

Tilman, D., 1999. Global environmental impacts of agricultural expansion: The need for sustainable and efficient practices. Proc. Natl. Acad. Sci. U. S. A. 96, 5995–6000. https://doi.org/10.1073/pnas.96.11.5995

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. Nature 418, 671–677. https://doi.org/10.1038/nature01014

Tittonell, P., 2019. Assessing resilience and adaptability in agroecological transitions. Microbiol. Res. 184, 102862. https://doi.org/10.1016/j.cageo.2011.03.003.As

Tittonell, P., 2014. Ecological intensification of agriculture-sustainable by nature. Curr. Opin. Environ. Sustain. 8, 53–61. https://doi.org/10.1016/j.cosust.2014.08.006

Tkaczuk, C., Król, A., Majchrowska-Safaryan, A., Nicewicz, Ł., 2014. The occurrence of entomopathogenic fungi in soils from fields cultivated in a conventional and organic system. J. Ecol. Eng. 15, 137–144. https://doi.org/10.12911/22998993.1125468

Torres, J.B., Bueno, A. de F., 2018. Conservation biological control using selective insecticides – A valuable tool for IPM, Biological Control. Academic Press. https://doi.org/10.1016/j.biocontrol.2018.07.012

Touw, A.J., Verdecia Mogena, A., Maedicke, A., Sontowski, R., van Dam, N.M., Tsunoda, T., 2019. Both biosynthesis and transport are involved in glucosinolate accumulation during root-herbivory in Brassica rapa. Front. Plant Sci. 10, 1653. https://doi.org/10.3389/FPLS.2019.01653

Tracy, E., 2015. The promise of biological control for sustainable Agriculture: a stakeholder-based analysis. J. Sci. Policy Gov. 5.

Tscharntke, T., Bommarco, R., Clough, Y., Crist, T.O., Kleijn, D., Rand, T.A., Tylianakis, J.M., Nouhuys, S. Van, Vidal, S., 2007. Conservation biological control and enemy diversity on a landscape scale. Biol. Control 43, 294–309. https://doi.org/10.1016/j.biocontrol.2007.08.006

Tscharntke, T., Karp, D.S., Chaplin-Kramer, R., Batáry, P., DeClerck, F., Gratton, C., Hunt, L., Ives, A., Jonsson, M., Larsen, A., Martin, E.A., Martínez-Salinas, A., Meehan, T.D., O'Rourke, M., Poveda, K., Rosenheim, J.A., Rusch, A., Schellhorn, N., Wanger, T.C., Wratten, S., Zhang, W., 2016. When natural habitat fails to enhance biological pest control – Five hypotheses. Biol. Conserv. https://doi.org/10.1016/j.biocon.2016.10.001

Tscharntke, T., Klein, A.M., Kruess, A., Steffan-Dewenter, I., Thies, C., 2005. Landscape perspectives on agricultural intensification and biodiversity - Ecosystem service management. Ecol. Lett. 8, 857–874. https://doi.org/10.1111/j.1461-0248.2005.00782.x

Tschumi, M., Ekroos, J., Hjort, C., Smith, H.G., Birkhofer, K., 2018. Predation-mediated ecosystem services and disservices in agricultural landscapes. Ecol. Appl. 28, 2109–2118. https://doi.org/10.1002/eap.1799

Tsiafouli, M.A., Thébault, E., Sgardelis, S.P., de Ruiter, P.C., van der Putten, W.H., Birkhofer, K., Hemerik, L., de Vries, F.T., Bardgett, R.D., Brady, M.V., Bjornlund, L., Jørgensen, H.B., Christensen, S., Hertefeldt, T.D.D., Hotes, S., Gera Hol, W.H.H., Frouz, J., Liiri, M., Mortimer, S.R., Setälä, H., Tzanopoulos, J., Uteseny, K., Pižl, V., Stary, J., Wolters, V., Hedlund, K., 2015. Intensive agriculture reduces soil biodiversity across Europe. Glob. Chang. Biol. 21, 973–985. https://doi.org/10.1111/gcb.12752

Tsunoda, T., Grosser, K., van Dam, N.M., 2018. Locally and systemically induced glucosinolates follow optimal defence allocation theory upon root herbivory. Funct. Ecol. https://doi.org/10.1111/1365-2435.13147

Turnock, W.J., Boivin, G., Whistlecraft, J.W.W., 1995. Parasitism of overwintering puparia of the cabbage maggot Delia radicum(L.) (Diptera:Anthomyiidae), in relation to host density and weather factors. Can. Entomol. 127, 535–542. https://doi.org/10.4039/Ent127535-4

Tylianakis, J.M., Romo, C.M., 2010. Natural enemy diversity and biological control: Making sense of the context-dependency. Basic Appl. Ecol. 11, 657–668. https://doi.org/10.1016/j.baae.2010.08.005

UK Government, 2020. Agriculture Bill 19, 1–94.

UK Government, 2018. A Green Future: Our 25 Year plan to improve the environment.

UN, 2015. Transforming our world: the 2030 Agenda for sustainable development. https://doi.org/10.1201/b20466-7

UNFCCC, 2016. FCCC/CP/2015/10/Add.1: Paris Agreement. Rep. Conf. Parties its twenty-first Sess. held Paris from 30 Novemb. to 13 December 2015 Add. 01194, 36.

Uphoff, N., Ball, A., 2006. Biological Approaches to Sustainable Soil Systems. Biol. Approaches to Sustain. Soil Syst. https://doi.org/10.1201/9781420017113

Uzman, D., Pliester, J., Leyer, I., Entling, M.H., Reineke, A., 2019. Drivers of entomopathogenic fungi presence in organic and conventional vineyard soils. Appl. Soil Ecol. 133, 89–97. https://doi.org/10.1016/j.apsoil.2018.09.004

Valverde, J., Reilly, K., Villacreces, S., Gaffney, M., Grant, J., Brunton, N., 2014. Variation in bioactive content in broccoli (Brassica oleracea var. italica) grown under conventional and organic production systems. J. Sci. Food Agric. https://doi.org/10.1002/jsfa.6804

van Dam, N.M., 2009. Belowground Herbivory and Plant Defenses. Annu. Rev. Ecol. Evol. Syst. 40, 373–391. https://doi.org/10.1146/annurev.ecolsys.110308.120314

van Dam, N.M., Raaijmakers, C.E., 2005. Local and systemic induced responses to cabbage root fly larvae (Delia radicum) in Brassica nigra and B. oleracea. Chemoecology 16, 17–24. https://doi.org/10.1007/s00049-005-0323-7

van der Putten, W.H., Bardgett, R.D., De Ruiter, P.C., Hol, W.H.G., Meyer, K.M., Bezemer, T.M., Bradford, M.A., Christensen, S., Eppinga, M.B., Fukami, T., Hemerik, L., Molofsky, J., Schädler, M., Scherber, C., Strauss, S.Y., Vos, M., Wardle, D.A., 2009. Empirical and theoretical challenges in aboveground-belowground ecology. Oecologia 161, 1–14. https://doi.org/10.1007/s00442-009-1351-8

van der Putten, W.H., Vet, L.E.M., Harvey, J.A., Wäckers, F.L., 2001. Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. Trends Ecol. Evol. 16, 547–554. https://doi.org/10.1016/S0169-5347(01)02265-0

van der Werf, H.M.G., Knudsen, M.T., Cederberg, C., 2020. Towards better representation of organic agriculture in life cycle assessment. Nat. Sustain. 3, 419–425. https://doi.org/10.1038/s41893-020-0489-6

van Diepeningen, A.D., de Vos, O.J., Korthals, G.W., van Bruggen, A.H.C., 2006. Effects of organic versus conventional management on chemical and biological parameters in agricultural soils. Appl. Soil Ecol. 31, 120–135. https://doi.org/10.1016/j.apsoil.2005.03.003

van Dijk, W.F.A., Lokhorst, A.M., Berendse, F., de Snoo, G.R., 2015. Collective agri-environment schemes: How can regional environmental cooperatives enhance farmers' intentions for agri-environment schemes? Land use policy 42, 759–766. https://doi.org/10.1016/j.landusepol.2014.10.005

van Eekeren, N., Boer, H., Bloem, J., Schouten, T., Rutgers, M., Goede, R., Brussaard, L., 2009. Soil biological quality of grassland fertilized with adjusted cattle manure slurries in comparison with organic and inorganic fertilizers. Biol. Fertil. Soils 45, 595–608. https://doi.org/10.1007/s00374-009-0370-2

van Geem, M., Harvey, J.A., Cortesero, A.M., Raaijmakers, C.E., Gols, R., 2015. Interactions Between a Belowground Herbivore and Primary and Secondary Root Metabolites in Wild Cabbage. J. Chem. Ecol. 41, 696–707. https://doi.org/10.1007/s10886-015-0605-7

Vandermeer, J., Armbrecht, I., De La Mora, A., Ennis, K.K., Fitch, G., Gonthier, D.J., Hajian-Forooshani, Z., Hsieh, H.-Y.Y., Iverson, A., Jackson, D., Jha, S., Jiménez-Soto, E., Lopez-Bautista, G., Larsen, A., Li, K., Liere, H., MacDonald, A., Marin, L., Mathis, K.A., Monagan, I., Morris, J.R., Ong, T., Pardee, G.L., Rivera-Salinas, I.S., Vaiyda, C., Williams-Guillen, K., Yitbarek, S., Uno, S., Zemenick, A., Philpott, S.M., Perfecto, I., 2019. The Community Ecology of Herbivore Regulation in an Agroecosystem: Lessons from Complex Systems. Bioscience 69, 974–996. https://doi.org/10.1093/biosci/biz127

Vanninen, I., Hokkanen, H., Tyni-Juslin, J., 1999. Attempts to control cabbage root flies Delia radicum L. and Delia floralis (Fall.) (Dipt., Anthomyiidae) with entomopathogenic fungi: laboratory and greenhouse tests. J. Appl. Entomol. 123, 107–113. https://doi.org/10.1046/j.1439-0418.1999.00315.x

Vanninen, I., Hokkanen, H., Tyni-Juslin, J., Tyni-Juslin, J., 1999. Screening of field performance of entomopathogenic fungi and nematodes against cabbage root flies (

Delia radicum l. and d. floralis (fall.); Diptera, Anthomyiidae). Acta Agric. Scand. Sect. B - Soil Plant Sci. 49, 167–183. https://doi.org/10.1080/09064719909362513

Varis, A.-L., 1967. Studies on the biology of the cabbage root fly (Hylemya brassicae Bouche) and the turnip root fly (Hylemya floralis Fall.). Ann. Agric. Fenn. 6, 1–13.

Vasseur, C., Joannon, A., Aviron, S., Burel, F., Meynard, J.M., Baudry, J., 2013. The cropping systems mosaic: How does the hidden heterogeneity of agricultural landscapes drive arthropod populations? Agric. Ecosyst. Environ. 166, 3–14. https://doi.org/10.1016/j.agee.2012.08.013

Vega, F.E., Goettel, M.S., Blackwell, M., Chandler, D., Jackson, M.A., Keller, S., Koike, M., Maniania, N.K., Monzón, A., Ownley, B.H., Pell, J.K., Rangel, D.E.N., Roy, H.E., 2009. Fungal entomopathogens: new insights on their ecology. Fungal Ecol. 2, 149–159. https://doi.org/10.1016/j.funeco.2009.05.001

Vervoort, M.T.W., Vonk, J.A., Mooijman, P.J.W., Van den Elsen, S.J.J., Van Megen, H.H.B., Veenhuizen, P., Landeweert, R., Bakker, J., Mulder, C., Helder, J., 2012. SSU ribosomal DNA-based monitoring of nematode assemblages reveals distinct seasonal fluctuations within evolutionary heterogeneous feeding guilds. PLoS One 7, e47555. https://doi.org/10.1371/journal.pone.0047555

Villani, M.G., Wright, R.J., 1990. Environmental influences on soil macroarthropod behavior in agricultural systems. Annu. Rev. Entomol. 35, 249–269. https://doi.org/10.1146/annurev.ento.35.1.249

Wagg, C., Bender, S.F., Widmer, F., Van Der Heijden, M.G.A., 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proc. Natl. Acad. Sci. U. S. A. 111, 5266–5270. https://doi.org/10.1073/pnas.1320054111

Waldner, T., Sint, D., Juen, A., Traugott, M., 2013. The effect of predator identity on post-feeding prey DNA detection success in soil-dwelling macro-invertebrates. Soil Biol. Biochem. 63, 116–123. https://doi.org/10.1016/j.soilbio.2013.03.030

Wall, D.H., Nielsen, U.N., Six, J., 2015. Soil biodiversity and human health. Nature 528, 69–76. https://doi.org/10.1038/nature15744

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Seta, H., Setala, H., van der Putten, W.H., Wall, D.H., 2004. Ecological Linkages Between Aboveground and Belowground Biota. Science (80-.). 304, 1629–1633.

Weber, D.C., Lundgren, J.G., 2009. Assessing the trophic ecology of the Coccinellidae: Their roles as predators and as prey. Biol. Control 51, 199–214. https://doi.org/10.1016/j.biocontrol.2009.05.013

Welbaum, G.E., Sturz, A. V., Dong, Z., Nowak, J., 2004. Managing Soil Microorganisms to Improve Productivity of Agro-Ecosystems. CRC. Crit. Rev. Plant Sci. 23, 175–193. https://doi.org/10.1080/07352680490433295

Wenninger, E.J., Vogt, J.R., Lojewski, J., Neher, O.T., Morishita, D.W., Daku, K.E., 2020. Effects of Strip Tillage in Sugar Beet on Density and Richness of Predatory Arthropods.

Environ. Entomol. 49, 33–48. https://doi.org/10.1093/ee/nvz135

Wezel, A., Bellon, S., Doré, T., Francis, C., Vallod, D., David, C., 2009. Agroecology as a science, a movement and a practice. A review. Agron. Sustain. Dev. 29, 503–515. https://doi.org/10.1051/agro/2009004

Wezel, A., Casagrande, M., Celette, F., Vian, J.F., Ferrer, A., Peigné, J., 2014. Agroecological practices for sustainable agriculture. A review. Agron. Sustain. Dev. 34, 1–20. https://doi.org/10.1007/s13593-013-0180-7

Wezel, A., Soboksa, G., McClelland, S., Delespesse, F., Boissau, A., 2015. The blurred boundaries of ecological, sustainable, and agroecological intensification: a review. Agron. Sustain. Dev. 35, 1283–1295. https://doi.org/10.1007/s13593-015-0333-y

Wilde, J. de., 1947. Onderzoekingen betreffende de koolvlieg (Chortophila brassica Bché) en zijn bestrijding.

Williams, C.D., Dillon, A.B., Girling, R.D., Griffin, C.T., 2013. Organic soils promote the efficacy of entomopathogenic nematodes, with different foraging strategies, in the control of a major forest pest: A meta-analysis of field trial data. Biol. Control 65, 357–364. https://doi.org/10.1016/j.biocontrol.2013.03.013

Willmott, D.M., Hart, A.J., Long, S.J., Richardson, P.N., Chandler, D., 2002. Susceptibility of cabbage root fly Delia radicum, in potted cauliflower (Brassica oleracea var. botrytis) to isolates of entomopathogenic nematodes (Steinernema and Heterorhabditis spp.) indigenous to the UK. Nematology 4, 965–970. https://doi.org/10.1163/156854102321122584

Wilson, C., Tisdell, C., 2001. Why farmers continue to use pesticides despite environmental, health and sustainability costs. Ecol. Econ. 39, 449–462. https://doi.org/10.1016/S0921-8009(01)00238-5

Winkler, K., Wäckers, F.L., Termorshuizen, A.J., van Lenteren, J.C., 2010. Assessing risks and benefits of floral supplements in conservation biological control. BioControl 55, 719–727. https://doi.org/10.1007/s10526-010-9296-8

Winqvist, C., Bengtsson, J., Aavik, T., Berendse, F., Clement, L.W., Eggers, S., Fischer, C., Flohre, A., Geiger, F., Liira, J., Pärt, T., Thies, C., Tscharntke, T., Weisser, W.W., Bommarco, R., 2011. Mixed effects of organic farming and landscape complexity on farmland biodiversity and biological control potential across Europe. J. Appl. Ecol. 48, 570–579. https://doi.org/10.1111/j.1365-2664.2010.01950.x

Woltz, J.M., Isaacs, R., Landis, D.A., 2012. Landscape structure and habitat management differentially influence insect natural enemies in an agricultural landscape. Agric. Ecosyst. Environ. 152, 40–49. https://doi.org/10.1016/j.agee.2012.02.008

Wu, S., Youngman, R.R., Kok, L.T., Laub, C.A., Pfeiffer, D.G., 2014. Interaction between entomopathogenic nematodes and entomopathogenic fungi applied to third instar southern masked chafer white grubs, Cyclocephala lurida (Coleoptera: Scarabaeidae), under laboratory and greenhouse conditions. Biol. Control 76, 65–73. https://doi.org/10.1016/j.biocontrol.2014.05.002

Zehnder, G., Gurr, G.M., Kühne, S., Wade, M.R., Wratten, S.D., Wyss, E., 2007. Arthropod pest management in organic crops. Annu. Rev. Entomol. 52, 57–80. https://doi.org/10.1146/annurev.ento.52.110405.091337

Zhang, H., Potts, S.G., Breeze, T., Bailey, A., 2018. European farmers' incentives to promote natural pest control service in arable fields. Land use policy 78, 682–690. https://doi.org/10.1016/J.LANDUSEPOL.2018.07.017

Zinger, L., Gury, J., Alibeu, O., Rioux, D., Gielly, L., Sage, L., Pompanon, F., Geremia, R. a, 2008. CE-SSCP and CE-FLA, simple and high-throughput alternatives for fungal diversity studies. J. Microbiol. Methods 72, 42–53. https://doi.org/10.1016/j.mimet.2007.10.005